MICROBIAL DYNAMICS IN THE POSTHARVEST SUPPLY CHAIN OF FRESH PRODUCE—AN OBSERVATIONAL STUDY AND SIMULATION MODEL FRAMEWORK

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Claire Elizabeth Zoellner

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MICROBIAL DYNAMICS IN THE POSTHARVEST SUPPLY CHAIN OF FRESH PRODUCE—AN OBSERVATIONAL STUDY AND SIMULATION MODEL FRAMEWORK

Claire Elizabeth Zoellner, Ph. D.

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The integrity of the supply chain of fruits and vegetables is important to public health and well-being. With expanding global trade, the issue of unsafe imported foods has become more acute and fresh produce remains a top food category for both number of foodborne illnesses and outbreaks. Prevalence of pathogens in the production environment, risks during harvest, cross-contamination in handling, and temperature fluctuations all suggest an opportunity for tracing the cumulative effects of these events on microorganisms on products from farm to retail. The goal was to demonstrate and describe microbial dynamics in the postharvest supply chain of fresh tomatoes. An observational study was conducted on tomatoes sampled from four locations of the postharvest supply chain from Mexico to USA and analyzed individually for microbial populations: aerobic mesophiles (APC), total coliforms (TC), generic *Escherichia coli* (EC), and yeasts/molds (YM). APC differed ($p<0.05$) from 1.9±1.1, 1.7±1.1, 2.3±1.1 and 3.5±1.4 log CFU/g at postharvest, packing, distribution and supermarket, respectively. TC was <1 log CFU/g at postharvest, increased at packing (0.7±1.0 log CFU/g), decreased in distribution (0.4±0.8 log

CFU/g) and increased in supermarkets (1.4±1.5 log CFU/g). Generic *E. coli* was not identified from TC in this supply chain. YM remained <1 log CFU/g, with the exception of 1.1±1.3 log CFU/g at supermarkets and tomatoes were not visibly spoiled. Next, to describe how and why the populations changed, the same microbial count data were used in mixed linear and logistic regression models to determine significant factors for concentration and prevalence, respectively. Location explained prevalence changes in TC and YM ($p<0.05$), while days-in-transit best explained concentration dynamics in all populations ($p<0.05$), with each additional day contributing 0.4-0.5 log CFU/g. Models illustrated supply chain microbial dynamics as certain locations increased or decreased prevalence and concentration depending on day and microorganism. With this, the Produce Supply Chain with Microbial Travelers, a modeling tool and graphical user interface, was developed to explore the relative impact of different contamination scenarios and intervention strategies on microbial behavior in the fresh tomato supply chain. These results provide data and a model framework which may be useful for future risk assessments.

BIOGRAPHICAL SKETCH

Claire Zoellner was born in St. Louis, MO and attended high school in New Lenox, IL. She has a B.S. in Food Science and Human Nutrition from the University of Illinois Urbana-Champaign. After participating in several R&D internships in the food industry and working in the food microbiology lab of Dr. Michael Miller at the University of Illinois, she decided to pursue a Ph.D. at Cornell University in Food Science and Technology with minors in Epidemiology and Systems Engineering. As a USDA National Needs Fellow, her coursework, research and the opportunity to work internationally with fresh produce growers in Mexico, have led her to focus on characterizing microbial dynamics of the postharvest supply chain of fresh produce.

To the real Doc Z., who always wanted a doctor in the family.

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PREFACE

With expanding global trade, the issue of unsafe imported foods has become more acute. Ensuring the safety of imported foods requires training a cadre of experts able to tackle the unique food safety risks of a globally integrated world. In response to this need of strategic national importance, a doctoral program was established at Cornell University, with support from the USDA National Needs Training Program, with the purpose of training future leaders in food safety, particularly in international issues. This program was designed to provide fellows with a range of food safety knowledge and specialized skills necessary for success in this area. The basic components included: a core curriculum of classes, short courses, seminars and training certifications; a multidisciplinary dissertation research project; an international experiential learning opportunity; and, regular participation in professional/scientific meetings. International experiential learning opportunities, in countries that are major US trade partners and/or developing countries, were identified for the doctoral fellows to participate in a combination of research and industry activities for up to six months. All fellows also participated in short term activities related to international food safety, in the US and internationally (India). The program will produce Ph.D. scholars uniquely prepared to undertake leadership roles in industry, government, regulatory agencies or academia, and tackle food safety challenges in a dynamic international context.

CHAPTER 1

INTRODUCTION AND JUSTIFICATION

The integrity of the supply chain of fresh fruits and vegetables is important to the health and well-being of consumers as well as to the industry's commerce. While healthy-eating initiatives across the globe, but particularly in the United States of America (U.S.A.), have attempted to influence consumption of fresh produce across the population, consumption of this combined food category has remained between 14-15% of the diet in USA since 1990. However, as consumption overall has gradually risen, this volume has proportionally increased and currently includes more varied products due to consumer demands, climate conditions, costs of production, and crop seasonality. For example, in 2013, consumption of fresh fruits and vegetables in the US was 43M metric tons, 33% of which was satisfied by imported products *(12)*. This level of consumption had remained the same despite reports that it has also remained at the top for both number of foodborne illnesses (19,932) and outbreaks (625) from 2004-2013 *(7)*. Although as a percentage of consumption fresh produce is one of the safest food categories, the risk of foodborne illness is still a top public health concern. Recent notable outbreaks include *Salmonella* Poona in cucumbers in 2015, recurring outbreaks of cyclosporiasis in cilantro from 2012-2015, *Salmonella* Typhimurium and Newport in cantaloupes in 2012, *Listeria monocytogenes* in cantaloupe in 2011 and apples in 2014, *Salmonella* Saintpaul in jalapeño and serrano peppers in 2008, and *E. coli* O157:H7 in spinach in 2006 *(7)*.

As fresh produce is grown and harvested in a highly variable environment, minimally processed and packaged, transported long distances, and sold in the open

fresh market, there are many opportunities for introduction or proliferation of microorganisms harmful to both the product (spoilage bacteria, yeasts and molds) and humans (pathogenic bacteria, parasites and mycotoxin-producing molds). Furthermore, the postharvest handling activities do not include a definitive inactivation step, such as thermal processing. Therefore, the supply chain is specifically designed to both preserve and monitor attributes of the product and its production environment.

From the outbreaks mentioned, production and handling environmental conditions and monitoring are not always sufficient preventive measures. Due to continued occurrence of foodborne illness, in 2011, the United States Food and Drug Administration passed the Food Safety Modernization Act to address the need for national regulations to control practices and risks associated with our food supply, especially fresh produce. Subsequent research and data needs have been identified to develop guidance and implementation resources for the industry, particularly in pathogen survival in soil amendments and on food contact surfaces.

Previous research on prevalence of pathogens in the production environment, contamination risks during harvest, cross-contamination in cutting and packing, along with temperature fluctuations during storage and distribution all suggest that a research opportunity exists to trace the cumulative effects of these events on microbial populations on products in their route from farm to retail. Therefore, the goal of this doctoral dissertation research was to describe the microbial dynamics of microorganisms in the postharvest supply chain of fresh produce as influenced by such handling operations, via an observational study and development of a mathematical

model framework. Of importance to both the observational study and mathematical model were the known food safety risks associated with fresh produce and existing models and data for risk assessments, both reviewed below. Understanding and quantifying these dynamics can facilitate further development of handling practices, sampling plans, intervention strategies and mathematical simulations, with the ultimate goal of preventing outbreaks of foodborne illness associated with fresh produce.

1.1 FRESH PRODUCE: A FOOD SAFETY RISK

Postharvest handling and technology of fresh fruits and vegetables have been designed to slow biological deterioration (senescence) and reduce losses from spoilage microorganisms in order to preserve product quality, especially since consumer decisions are based on flavor, ripeness and appearance *(6)*. However, the postharvest steps taken must also function to prevent or minimize the presence of human pathogens that may cause foodborne illness upon consumption. The pathogens most commonly associated with fresh produce can come from soil and the growing or packing environments, but more abundant are those from enteric environments—the intestinal tracts and feces of animals and workers. Therefore, contamination can and has occurred during growth, harvest, packing, distribution and final preparation stages of the postharvest supply chain via a variety of mechanisms such as feces, sewage, water, soil, insects, animals, machinery and human handling.

Table 1.1 below outlines the pertinent pathogens historically associated with fresh produce that should be considered in food safety plans and risk assessments. The differences in infective dose and incubation period create clinical challenges to

outbreak investigation and mitigation. Moreover, the variety of produce items associated with one pathogen (i.e., *Salmonella)* or the variety of pathogens associated with a single produce item (i.e., apple juice, lettuce) reinforce the need for thorough risk assessments and varied intervention strategies. The proposed mathematical model framework in this dissertation was purposely designed to be flexible enough to capture physiological differences of pathogens in transfer, growth, survival, and susceptibility to wash methods in order to test such interventions for different pathogen-produce item risk scenarios.

Table 1.1 Pertinent pathogens historically associated with fresh produce *(11)*

Pathogen	Infectious dose (number of cells)	Incubation Period	Produce item(s)	Source(s)
		BACTERIA		
Bacillus cereus	intoxication: growth (10^5-10^8) and toxin production	diarrheal: 6-15 hours	seed sprouts	decaying organic matter, fresh water, vegetables and fomites, intestinal tract of invertebrates
Clostridium botulinum	intoxication: growth and toxin production in food	12-36 hours	cabbage, dried garlic in oil	soil, lakes, streams, decaying vegetation, reptiles
Enterohemorrhagic *Escherichia coli* (EHEC)	10 to 100 (O157:H7), slightly higher for other serotypes	2-5 days	apple juice, lettuce mix, alfalfa sprouts, cabbage, cucumbers, tomatoes, spinach	animal feces (cattle, deer and human), cross-contamination from raw meat
Listeria monocytogenes	1,000	1 day to 5+ weeks	cabbage, cantaloupe, apples, shredded lettuce	soil, food processing environments
***Salmonella* spp**	10 to 1,000	18-72 hours	cantaloupe, mangoes, tomatoes, seed sprouts, watermelon, orange juice, apple juice, leafy greens, cucumbers	animal and human feces, raw meat, poultry, eggs
***Shigella* spp.**	10	1-3 days	green onion, parsley, lettuce	human feces
Vibrio cholerae	10^3-10^8	2-3 days	coconut milk	human feces
		PARASITES		
Cyclospora cayentanensis	unknown, but suspected low (10-100 oocysts)	1-11 days	raspberries, lettuce, basil, cilantro	domestic animals, feces of chickens, intestines of mammals and birds
Cryptosporidium parvum	30 oocysts	1-12 days	apple juice, green onions	animal and human feces
		VIRUSES		
Hepatitis A	10 to 50	25-30 days	lettuce, frozen raspberries, strawberries, tomatoes	human feces and urine
Norovirus	unknown	12-48 hours	melon, green salad, celery	human feces, vomitus

The potential of such pathogens to be present at the time of consumption in levels sufficient to cause illness is what makes fresh produce a food safety risk and public health hazard. Pathogens may contaminate and survive on produce items and the infectious dose (minimum number of microorganisms necessary to cause infection in the host) is typically low (Table 1.1). Generally, pathogenic bacteria will survive but will not divide and multiply on the uninjured outer surface of fresh fruits and vegetables, especially if the humidity is high, due to protective barriers innate to the plant physiology (i.e., cell walls and wax layers) *(10)* and the lack of enzymes required for breaking down these protective barriers to release nutrients necessary for growth. Therefore, in some cases pathogen levels will decline on this outer surface depending on the organism, product and conditions. In other cases, microorganism survival is enhanced under refrigerated conditions or when surface tissue is damaged mechanically or by other plant pathogens (bacteria or fungi). Furthermore, some pathogens are psychrotrophic and can grow in cold temperatures, most notably *Listeria monocytogenes.* So while temperature control can be a risk control strategy, management of initial contamination sources is more effective.

1.1.1 Indicator Microorganisms

Levels of foodborne pathogens are not routinely tested for presence in the production environment or on food products because of their low presence, long detection and enumeration process, and untimely indication of a contamination event. Instead, produce growers and handlers are encouraged to utilize Good Agricultural Practices (GAPs) and other similar voluntary grower programs focused on monitoring the risk areas of water, soil amendments, wildlife, workers and food contact surfaces

on farms in order to prevent and control such sources of contamination. Furthermore, a written food safety plan encompasses the pertinent background and production practice information required to appropriately assess the unique risks faced on a farm. These plans also serve as record-keeping documentation for implementation of controls to mitigate identified risks. In compliance with certifications for food safety practices, annual audits are often conducted and require some environmental testing for indicator microorganisms.

Determination of the microbiological quality of a food product or production environment may be done using indicator microorganisms *(13)*. The type of indicator used may vary according to the product, environmental sample or pertinent pathogen of interest. In general, there are indicators of quality and indicators of safety. Indicators of quality are typically total yeasts and molds and aerobic mesophilic or total plate count, each of which at certain levels may be used to assess the current organoleptic quality of a product or the sanitary quality of the handling environment, and predict the shelf life of a product. Typically the cut-off of at least 6-8 log CFU/g aerobic plate count or yeasts and molds is required for visible spoilage *(4)*.

Indicators of safety refer to the increased likelihood of enteric human pathogens that may cause foodborne illness. These indicators are useful as they are more readily and easily detectable than pathogens, and also have characteristics that relate to the presence, growth, survival and levels of pathogens in food products and the environment. For example, shown in Figure 1.1 are the most common safety indicators and their relationship to several pertinent enteric pathogens associated with causing foodborne illness upon consumption of fresh produce.

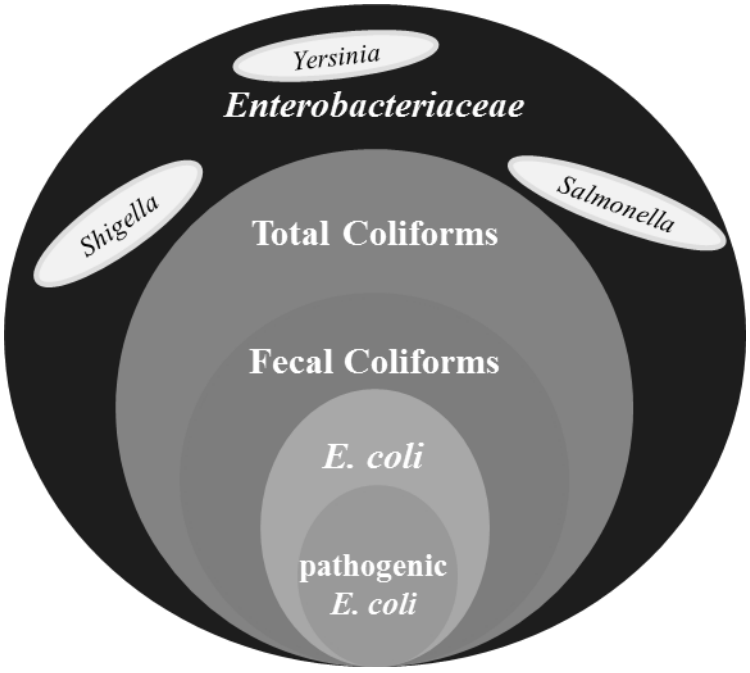

Figure 1.1 Relationship between common food safety indicator microorganisms and enteric human pathogens.

Enterobacteriaceae is the family of facultatively anaerobic, generally mesophilic, gram-negative, straight bacilli, and are able to ferment glucose to produce acid. Foodborne genera of this family include: *Citrobacter, Enterobacter, Erwinia, Escherichia, Klebsiella, Salmonella, Serratia, Shigella,* and *Yersinia,* among others. Within this family is Total Coliforms, named so based on biochemical reactions. This group represents bacteria which are aerobic, gram-negative, non-sporeforming rods that ferment lactose, forming acid and producing gas within 48 hours at 35°C. Fecal coliforms are those coliforms which can ferment lactose, forming acid and producing gas within 48 hours but at slightly higher temperatures, 44.5-45.5°C, and in EC broth. This group may encompass strains of *Escherichia coli, Klebsiella pneumoniae, Enterobacter* spp., and *Citrobacter freundii*. Biochemical testing, such as Indole,

Methyl Red, Voges Proskauer, and Citrate or production of β-glucuronidase, of fecal coliform colonies will confirm generic *E. coli*.

It is important to note that *Enterobacteriaceae* and coliforms may also encompass microorganisms of non-enteric sources *(13)*, so their presence does not always suggeset contamination with fecal matter and their use as an indicator is often disputed. In fact, *Enterobacteriaceae* is among the most abundant bacterial families on fresh fruits and vegetables and is considered in the native microbiota. Leff and Fierer *(14)* characterized the diversity of bacterial communities associated with the surfaces of fresh fruits and vegetables purchased from retail and showed *Enterobacteriaceae* to be the most abundant (20-90%) family on bean sprouts, spinach, lettuce, tomatoes, peppers, alfalfa sprouts and strawberries.

1.2 QUANTITATIVE MICROBIAL RISK ASSESSMENT (QMRA): THE POTENTIAL FOR A SOLUTION

Modeling is a technique used to represent the behavior of a system and is needed by government and industry to reduce the time and cost associated with assessing food safety. The fields of predictive microbiology and quantitative microbial risk assessment (QMRA) in food and food systems have become increasingly active. Specifically, these predictive microbial models use mathematical expressions to represent the number, concentration or prevalence of microorganisms as a function of intrinsic (i.e., pH, water activity) or extrinsic (i.e., temperature) factors in food products or processes. These models then fit into the QMRA framework, which includes hazard identification, exposure assessment, risk characterization, dose-response and risk management. The output is usually an estimate of illnesses

associated with consumption of the contaminated product. Currently, QMRA models of food systems include journal publications *(6, 8, 16)*, open access software and databases, and federal tools, and they span several categories of models: deterministic primary and secondary models, probabilistic/stochastic simulation, and risk ranking, which are reviewed in Table 1.2.

Table 1.2 Description of selected current software for microbial modeling in food *(23)*

Software (date of creation)	Link	Accessibility	Description	Features
		DETERMINISTIC		
Baseline (2012)	www.baselineapp.com	free, web-based	Using predictive models from the literature, the growth and inactivation of 5 pathogens are simulated in several food matrices.	growth and inactivation prediction
ComBase (2004)	http://www.combase.cc	free, web-based	Database of growth and inactivation of 15 microorganisms in media and food for fitting and simulation.	database, growth and inactivation fitting and prediction
Pathogen Modeling Program (1991)	http://pmp.errc.ars.usda.gov/PMPOnline.aspx	free, web-based	Package of models for predicting growth, heat inactivation, survival and transfer of pathogens in media, meat or seafood.	database of primary and secondary models
		PROBABILISTIC		
Quantitative Produce Risk Assessment Model (ongoing)	under development	not yet released	Agent-based, virtual laboratory to model specific practices and risk factors for contamination of fresh produce at individual farm or processing facility level.	risk assessment, sampling patterns
microHibro (2011)	www.microhibro.com	free, web-based	Fits a primary model to experimental data for parameterization. Risk assessment module allows user to design and simulate scenarios leading to final concentration at consumption.	specific to produce, database, growth prediction, risk assessment
GroPIN (2013)	www.aua.gr/psomas/gropin	free, downloadable	Simulates the behavior of 66 microorganisms in food matrices from a database of predictive models or new data—allows for Monte Carlo simulations.	database, growth and inactivation prediction
FDA-iRISK (2012)	https://irisk.foodrisk.org	free, web-based	Quantitatively compares and ranks risks of food/hazard combinations based on seven process elements including dose-response.	database, risk assessment
		RISK RANKING		
Produce Risk Ranking Tool (2009)	https://foodrisk.org/exclusives/rrt/	free, downloadable	Quantitatively compares and ranks risks of produce/hazard combinations based on weighted scores from nine criteria.	database of outbreak data

In conducting a risk assessment, what is desired is a quantitative evaluation of the source and occurrence of contamination, the behavior of the contamination in the

given conditions, and the characterized human response upon consumption. From the existing tools mentioned above, user inputs and risk ranking suggest the occurrence of contamination in specific foods, while the deterministic and probabilistic models indicate the general behavior (growth, inactivation, survival, transfer) of microorganisms given environmental parameters. Some tools use this final concentration in a dose-response model to quantify the probability and severity of illness for use in policy and decision-making. Many of these tools rely on databases of similar experimental data from culture-based behavior, are focused on a specific commodity or process, and lack flexibility for multiple sources of potential contamination. What is needed is a model specific to fresh produce, that includes the possibility of contamination at multiple points, and that traces microbial behavior beyond growth and inactivation.

Beyond the modeling software listed above, research on food supply chains has focused on tactical planning to maximize profitability during production and distribution of perishable goods *(1, 2, 24)*, failure modes *(15, 22)*, cross-contamination scenarios during food processing *(3, 16, 17, 19, 20)*, and comparisons of different risk modeling approaches *(9, 21)*. The same conclusion can be drawn from this body of literature: that a gap exists in the consideration of multiple microbial risks throughout production and distribution of minimally processed products, such as fresh produce. However, several recent publications also provide interesting approaches to modeling such microbial behavior in other food systems that can be adapted to fresh produce. For example, as cross-contamination has been critical in meat, poultry and deli operations, similar methods in these models can be applied to fresh produce packing

and distribution. Still, the field of QMRA has been challenged by lack of data, limitations of the predominant concepts, unique complexity of food products, and diversity of supply chain risks. As a conclusion, gaps exist specifically in the areas of testing the effectiveness of intervention methods, spatial and temporal variation in microbial populations, and pathogen prevalence, transfer, disposition and survival.

The supply chain model proposed in this dissertation was derived from and builds upon the evolving QMRA exposure assessment concept of segmenting the supply chain into generic processes that encompass microbial behavior, called Modular Process Risk Modeling (MPRM) *(17,18)*. It is a tool to identify the impact of intervention strategies along the supply chain, and not to quantify the estimate of risk upon consumption, per se. The modular, linear structure has been expanded to include a network of nodes that better explains today's complex supply chain of fresh produce. This is a preliminary deterministic framework that uses simulation to step through a series of mathematical models describing microbial behavior in a customizable fresh produce supply chain. The proposed dynamic contamination model was designed to make quantitative microbial models encompass the variety of behavior in a supply chain, while maintaining a diagrammatic, user-friendly interface for customized scenarios.

The aim of this doctoral research has been to quantify and describe microbial populations in a supply chain in order to move beyond existing mathematical modeling tools. The dissertation is organized in chapters to address the progression of observations to reach this end. First, in an attempt to study and collect data on such phenomena, an observational study was designed to sample fresh tomatoes from their

supply chain from Mexico to the USA to observe that *microbial populations change on produce in a supply chain* (Chapter 2). Statistical analysis of this study was an approach to conclude that *microbial populations change in prevalence and concentration depending on supply chain factors* (Chapter 3). Qualitative and quantitative data were considered in development of the simulation model framework to illustrate that *microbial behavior in supply chains can be generalized and mathematically modeled* (Chapter 4). And, finally to demonstrate the utility of such a model, and *what can be learned from simulations*, PSCMT modeling tool was applied to the observed supply chain to test parameters related to management decisions (Chapter 5).

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CHAPTER 2

MICROBIAL DYNAMICS OF INDICATOR ORGANISMS ON FRESH TOMATOES IN THE SUPPLY CHAIN FROM MEXICO TO THE USA

ABSTRACT

Quality and safety of fresh produce are important to public health and maintaining commerce between Mexico and USA. While preventive practices can reduce risks of contamination and are generally successful, the variable environment of the supply chain of fresh produce can be suitable for introduction or proliferation of pathogenic microorganisms. As routine surveillance of these pathogens is not practical, indicator microorganisms are used to assess the sanitary conditions of production and handling environments. An opportunity exists to use indicators on fresh produce to measure how handling and transport from field to market may affect microbial populations that contribute to their quality or safety. The objective was to quantify indicator microorganisms on tomatoes sampled along the supply chain during the harvest year, in order to observe the levels and changes of populations at different locations. Roma tomatoes (n=475) were taken from the same lots (n=28) at four locations of the postharvest supply chain over five months: at arrival to and departure from the packinghouse in México, at the distribution center in Texas, and at retail in USA. Samples were analyzed individually for four microbial populations: aerobic plate count (APC), total coliforms (TC), generic *Escherichia coli*, and yeasts and molds (YM). APC population differed (p<0.05) from 1.9±1.1, 1.7±1.1, 2.3±1.1 and 3.5±1.4 log CFU/g at postharvest, packing, distribution center and supermarket, respectively. TC populations were <1 log CFU/g at postharvest, increased at packing

(0.7±1.0 log CFU/g), decreased in distribution (0.4±0.8 log CFU/g) and increased in supermarkets (1.4±1.5 log CFU/g). Generic *E. coli* was not identified from coliform populations in this supply chain. YM populations remained <1 log CFU/g, with the exception of 1.1±1.3 log CFU/g at supermarkets and tomatoes were not visibly spoiled. The levels reported from this pilot study demonstrated the dynamics within populations as influenced by time and conditions in one supply chain during a harvest year, while the large variances in some locations indicate opportunities for improvement. Overall, packinghouse and supermarket locations were identified as crucial points to control microbial safety risks.

Keywords: tomatoes, indicator microorganisms, postharvest, supply chain, safety

2.1 INTRODUCTION

The supply chain of fresh produce from Mexico to the United States is important to the health and well-being of consumers and to the industry's commerce. A wide variety of fresh fruit and vegetable products traverse this border each year, totaling 13 billion pounds worth over 6.2 billion US dollars. While there is domestic production of fresh tomatoes, imports account for about half of US consumption and originate mainly from Mexico (85%) and Canada (13%). The imported fresh tomato category includes the following varieties: cherry (2%), grape (4%), round (16%), Roma (37%), and hothouse/greenhouse (41%). Mexico holds 71% market share of imported hothouse tomatoes and 99% market share of imported Roma tomatoes *(27, 28)*. Regardless of their origin, fresh tomatoes are generally hand-picked and consumed raw, making both the quality and safety of these products essential for maintaining this industry. As such, the supply chain is specifically designed to both preserve and monitor attributes of the product and its production environment. Programs such as Good Agricultural Practices (GAPs) are in place to reduce the risk of product degradation and contamination in production, harvest and handling environments. Third party auditing groups serve to verify the legitimacy of such practices and records within each operation. Postharvest handling practices specific to quality include culling damaged products after harvest, washes, sanitizer treatment, storage and transportation under controlled atmosphere conditions, and visual inspection upon receipt of the product at distribution and retail centers.

Still, there exist processes and conditions suitable for introduction, survival or

growth of microorganisms that can affect produce safety or quality as it travels from field to the point of sale *(1, 6, 7, 8, 9, 14, 18, 20, 21, 26).* Fifteen multi-state outbreaks (1959 illnesses) of *Salmonella* in the USA between1990-2010 were associated with round (69%), Roma (23%), and grape (8%) tomatoes. Although epidemiological studies linked cases to consumption domestically produced tomatoes in restaurants, traceback investigations into contamination sources were often complicated by the web of grower, packers, distributers and retailers that handled the product *(4).* Pathogen surveillance in the final product is not necessarily a practical or successful measure for a farm, packinghouse or distribution center to use in order to assess the quality or safety of a fresh produce item. Depending on the microorganism, prevalence may be low, results can take up a considerable amount of the product's shelf life, the entire lot must be held and possibly removed from commerce, and the results may not indicate the source of the problem.

Instead, indicator microorganisms, such as *Enterobacteriaceae*, coliforms and generic *Escherichia coli*, may be used in assessments of the overall quality of a product and the hygienic conditions present in its production and handling environments *(16).* In this study, indicator organisms were used for understanding the potential influences of the supply chain on microbial populations on fresh produce. Roma tomatoes produced in Mexico and exported to several retail markets in different states of the USA were followed to study these hypothesized population dynamics, with the objective of quantifying the magnitude of changes due to conditions along the supply chain during one harvest year.

2.2 MATERIALS AND METHODS

2.2.1 The supply chain and sampling locations

Refer to Figure 2.1 for the overall sampling design. Roma tomatoes were produced on a farm located in the state of Nuevo León, México. This farm utilized protected agriculture systems of greenhouses and shade houses on forty-two acres, which corresponded to different lot codes of the final products. Drip fertigation of plants drew water from deep wells on-site. Tomatoes were hand-harvested, with stems removed, into plastic containers. Tomatoes were transported to a packinghouse located within the same farm, where they were spray-washed with chlorinated water and brush rollers. 150 ppm total chlorine was measured and maintained in the wash water every hour using test strips (Diken International, Monterrey, Mexico) and completely changed every 4 hours or 24 pallets, whichever came first. After washing and sanitizing, tomatoes were forced-air dried on foam rollers, conveyed through sorting and hand-packed into boxes.

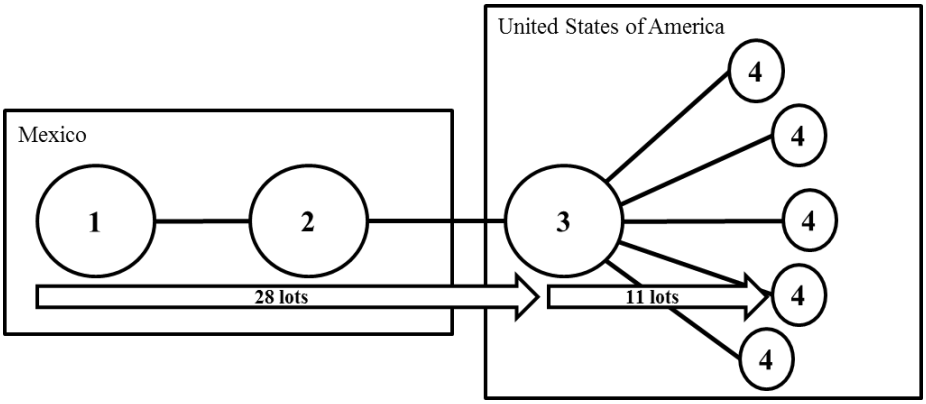

Figure 2.1 Schematic of the supply chain sampling from Nuevo Leon, Mexico to several supermarkets in the USA. The following locations were sampling points for a total of 28 lots of tomatoes: (1) Arrival to packinghouse (n=130); (2) End of the packing line (n=130); (3) Distribution center storage room (n=144). Of those 28 lots, only 11 lots were recovered in the final location: (4) Retail supermarkets in the United States (n=71).

The first sampling location was upon arrival to the packinghouse after harvesting, where tomato samples were taken with gloved hands from their plastic harvesting containers (referred to as "harvest"). The second sampling location was after the washing and sorting steps and immediately prior to boxing, where tomato samples were taken by the workers ("packing" or "packinghouse"). The same day, palletized boxes were loaded onto refrigerated trailer trucks for transportation to and storage in a distribution center in southern Texas, USA. The third sampling location ("distribution") was palletized boxes in the cold storage room (9-10°C) of the distribution center, with gloved hands after several days of storage and prior to shipment to clients. Tomato boxes were sold based on size and color to retail supermarkets in Texas, North Carolina, Minnesota, and Michigan, USA. The final sampling location was the point of sale in supermarkets or in one occasion from the supermarket storage room ("supermarket"), again with gloved hands. In all instances, the same lot codes designated by the farm and displayed on each box were followed through the supply chain for a total of 28 different lots through distribution and 11 of those lots through retail, over one production season.

Tomato samples (4-10 fruits) were taken individually in Ziploc bags from each lot code depending on the sampling location and were maintained on ice until individual analysis (within 48 hours). A total of 475 tomatoes were taken throughout the supply chain: 130 tomatoes at postharvest, 130 at packing, 144 at the distribution center, and 71 from five different supermarkets. Difficulty in traceability of lots explains the different number of samples at each location along the supply chain.

Control samples (n=30 fruits) were taken from the harvest location and

maintained at 10-12°C, 90% relative humidity (RH) for up to ten days. Similarly, additional control samples (n=30 fruits) were taken immediately after the chlorinated wash and maintained 10-12°C, 90%RH for up to ten days. These tomatoes did not travel the supply chain, but were maintained in storage conditions typical for maximizing postharvest quality during distribution and storage *(22)* and analyzed for microbial indicators every other day.

2.2.2 Microbiological analyses

Tomato samples were analyzed at the Autonomous University of Nuevo León (San Nicolas, NL México) or Cornell University (Ithaca, NY USA) following the same protocol: Into the bag containing the tomato sample (83±20 g), an equal volume to weight ratio of 0.1% peptone water was added and the tomato surface was washed by gentle rubbing for 1 minute. All enumeration methods followed the pour plate technique using 1 ml, 100 µl and 10 µl of this rinse water. For aerobic plate count, serial dilutions were plated in duplicate using Standard Methods Plate Count Agar (BD Bioxon, Cuautitlán, México; Alpha Biosciences, Maryland USA) and incubated for 48 hours at 35°C. For total yeasts and molds, serial dilutions were plated in duplicate using Potato Dextrose Agar (Oxoid, Monterrey, México; Alpha Biosciences, Maryland USA) containing 10% tartaric acid and incubated for 5 days at 25°C. For total coliforms and generic *Escherichia coli*, serial dilutions were plated in duplicate using one layer of Violet Red Bile Agar (BD Difco, Maryland USA; Hardy Diagnostics, Santa Maria, California USA) (VRBA) and a second layer of Violet Red Bile Agar containing 100µg/ml 4-methyl-umbelliferyl-β-D-glucuronide (BD Difco, Maryland USA; Hardy Diagnostics, Santa Maria, California USA) (VRBA+MUG)

and incubated for 24 hours at 35°C. VRBA+MUG plates were first examined under illumination and purple-red colonies with white precipitate were counted as total coliforms and representative colonies were confirmed by lactose fermentation and gas production using incubation for 24-48-h at 35°C in Brilliant Green Lactose Bile (BD Bioxon, Cuautitlán, México; Hardy Diagnostics, Santa Maria, California USA) (BGLB) broth containing a Durham tube. VRBA+MUG plates were also examined under longwave UV light (365-nm) for the presence of colonies with blue fluorescence, indicative of *E. coli*. The limit of detection was 1 CFU/g tomato. Samples with no detectable colonies were assigned this value for statistical analyses and the number of negative samples was reported. The results for the indicator microorganisms were reported as geometric means of $\log_{10}$ CFU/g tomato.

2.2.3 Statistical analyses

Analysis of variance in geometric means within each indicator population was performed using SAS 9.3 (PROC GLM, SAS Institute Inc., Cary, NC, USA). In control tomatoes, log CFU/g was analyzed over 10 days of controlled storage. In sampled tomatoes, log CFU/g was analyzed over the supply chain locations and multiple comparisons across the locations were performed using the Tukey-Kramer Honestly Significant Difference (HSD) test, with $\propto$=0.05. . Results for tomatoes from the supply chain locations are illustrated using boxplots to provide maximum, minimum, mean, median and interquartile range (IQR) of the $\log_{10}$CFU/g values. Lastly, log CFU/g results of indicator populations at the supermarket location were analyzed according to the day postharvest to assess differences in supply chain length (HSD, $\propto$=0.05).

2.3 RESULTS

2.3.1 Control Tomatoes

The control tomatoes were sampled at arrival to the packinghouse on the day of harvest ("harvest") and immediately from the packing line after washing and sanitizing ("washed") and were stored in controlled temperature and relative humidity for 10 days. Tomatoes sampled at harvest showed an initial level of aerobic plate count (APC) of 1.8±0.3 log CFU/g. Tomatoes sampled at days 2, 4, 6, 8 and 10 during controlled storage had APC levels of 1.2±0.3, 1.7±0.4, 1.7±0.7, 2.1±0.6 and 1.3±0.6 log CFU/g, respectively (Figure 2.2). This change in APC over 10 days was not significant ($p>0.05$). The control tomatoes taken after spray washing with chlorinated water showed an initial level of APC of 1.0±0.8 log CFU/g, which was a significant reduction compared to harvested tomatoes ($p<0.05$). APC on the washed tomatoes sampled at days 2, 4, 6, 8 and 10 during controlled storage were 0.4±0.6, 0.4±0.5, 0.9±0.2, 1.5±0.5, and 0.6±0.5 log CFU/g, respectively, and did not show a significant change ($p>0.05$). Four out of five control tomatoes had undetectable levels of total coliform (TC) sampled at harvest, with one tomato containing 1.4 log CFU/g. Tomato samples immediately after washing with chlorine did not contain detectable TC (n=5/5). Throughout 10 days in controlled storage, TC populations remained below detection on harvested (n=25/25) and on washed tomatoes (n=24/25), with the exception of one tomato containing 1.4 log CFU/g on day 10. None of the TC colonies were identified as generic *E. coli*. YM populations were not detected on harvested (n=5/5) or washed tomatoes (n=5/5) at day 0 or throughout 10 days of controlled storage for either treatment (n=25/25).

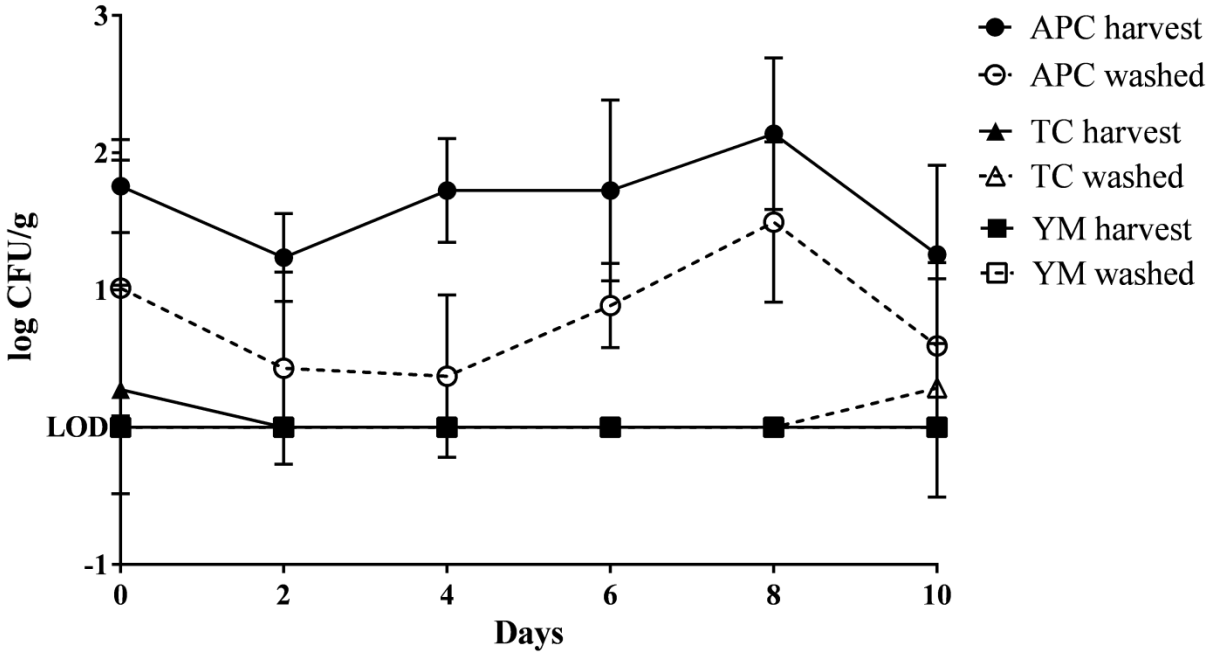

Figure 2.2 Aerobic plate counts, total coliforms, and yeasts and molds on tomatoes taken at harvest and post-washing and stored for 10 days at 10-12°C and 90% relative humidity.

2.3.2 Aerobic plate count

Shown in Figure 2.3 is the change in aerobic plate counts (APC) on tomatoes sampled during postharvest events of the supply chain. The difference between the harvested and sold product was 1.6 log CFU/g increase (95% CI= (1.1, 2.0), $p<0.05$), indicating either significant growth or introduction after departure from the farm/packinghouse. From production and harvest, the initial level of APC on the tomatoes at arrival to the packinghouse was 1.9±1.1 log CFU/g. The maximum load of APC per tomato arriving to the packinghouse was found to be 4.5 log CFU/g and six samples (5%) had undetected levels (<1 CFU/g). On the same day, after washing and packing, the level of APC remained statistically the same ($p>0.05$) at 1.7±1.1 log

CFU/g with a maximum detected load of 4.4 log CFU/g and seven (5%) negative samples (<1 CFU/g). The population increased (p<0.05) after transportation to and storage at the distribution center to 2.3±1.1 log CFU/g tomato. There, the maximum level detected was 4.7 log CFU/g and three tomatoes (2%) had no detectable APC. Lastly, the APC level was highest by the end of the supply chain (p<0.05) at the supermarket at 3.5±1.4 log CFU/g with a maximum of 5.8 log and only one sample (1%) below detection. The consistent standard deviation in the first three locations suggested a natural variability in this population that was altered during retail conditions.

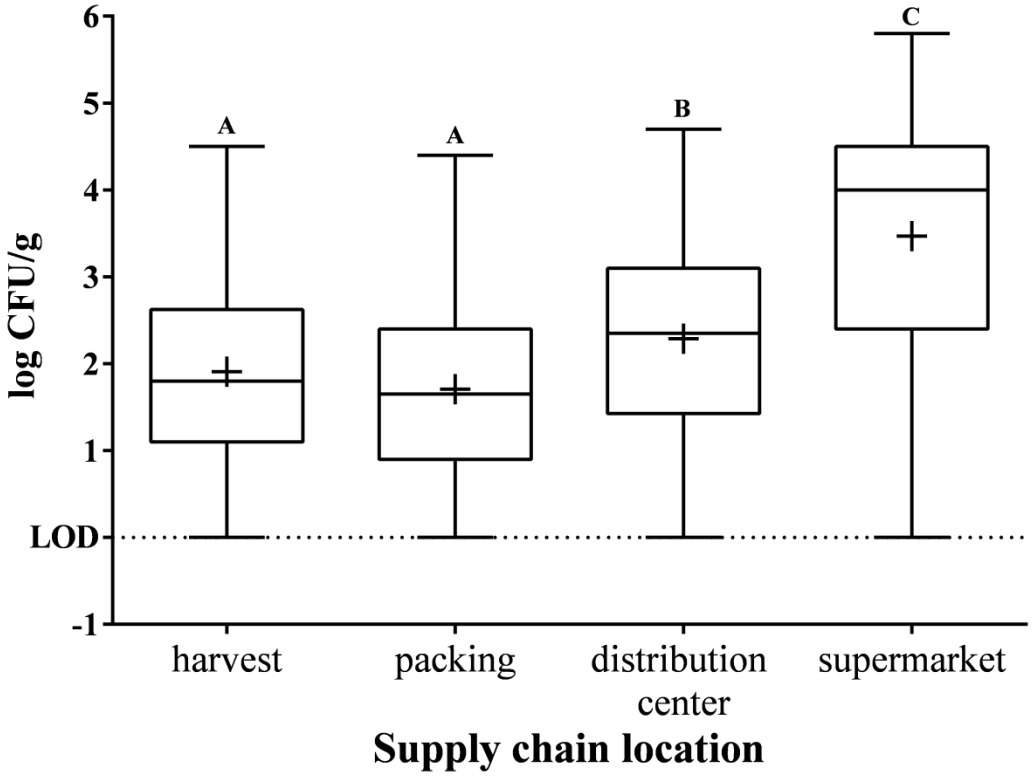

Figure 2.3 Aerobic plate counts (log CFU/g) on tomatoes sampled from the postharvest supply chain. +: location geometric mean. LOD: limit of detection. A: boxes-and-whiskers marked with the same letter are not significantly different (Tukey-Kramer HSD, α=0.05).

2.3.3 Total coliforms

The total coliform (TC) population on tomatoes during the postharvest supply chain (Figure 2.4) differed from APC with regard to observed levels, changes and non-detected sample numbers at harvest, packing and distribution locations. Of the TC population, none were identified as generic *Escherichia coli*. 85% of tomatoes arriving at the packinghouse from harvest were below the detection limit. When TC was detected from harvest, levels ranged from 0.2-3.7 log CFU/g. After the tomatoes were washed and conveyed through sorting to packing, the TC level significantly increased ($p<0.05$) to 0.7±1.0 log CFU/g, with 52% of samples below the detection limit and a maximum level of 4.0 log CFU/g. TC decreased during distribution, with 70% of samples below detection, and was not different from the harvest levels (0.1-3.9 log CFU/g). TC increased at the supermarket location to 1.4±1.5 log CFU/g, where 3.9 log CFU/g was the maximum and 45% of tomato samples were below detection. The overall difference in total coliform population from harvested tomatoes to sold tomatoes was an increase by 1.2 log CFU/g (95% CI= (0.9, 1.6), indicating significant postharvest change ($p<0.05$). The standard deviations, ranging from 0.6-1.5 across the supply chain locations, and high number of non-detected samples for TC population suggested areas for future sampling and intervention.

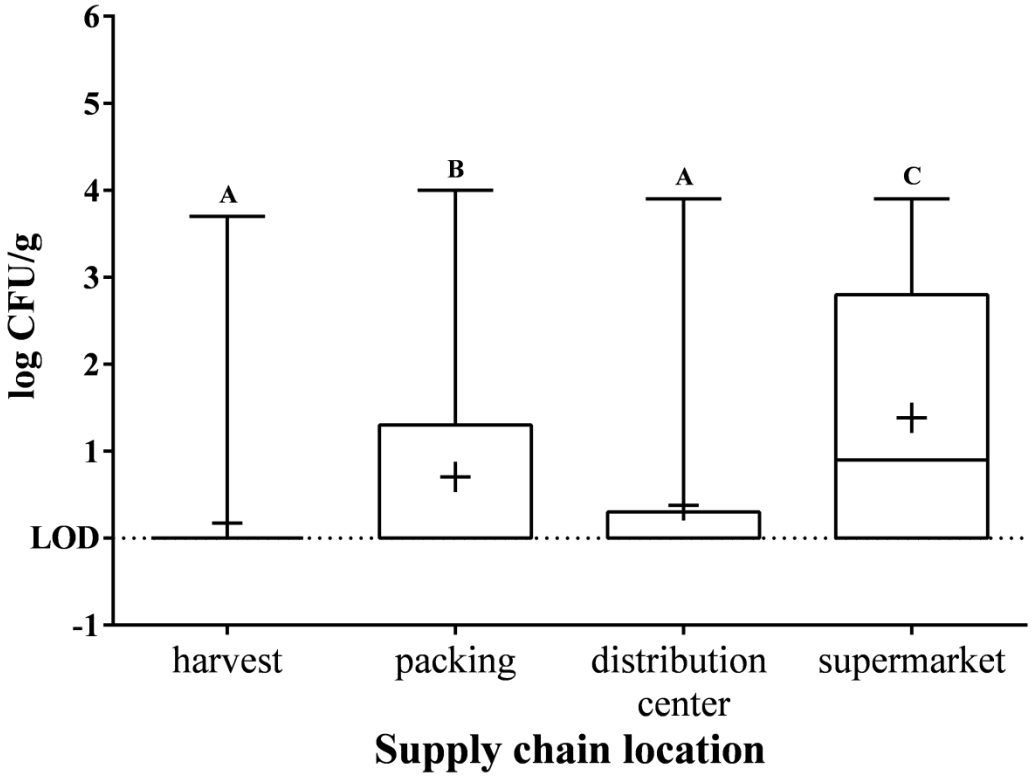

Figure 2.4 Total coliforms (log CFU/g) on tomatoes sampled from the postharvest supply chain. +: location geometric mean. LOD: limit of detection. A: boxes-and-whiskers marked with the same letter are not significantly different (Tukey-Kramer HSD, α=0.05).

2.3.4 Yeasts and molds

The yeast and mold (YM) population remained <1.0 log CFU/g from harvest through packing and distribution, at 0.4±0.6, 0±0.1, and 0.2±0.3 log CFU/g, respectively. The YM level increased to 1.1±1.3 log CFU/g by the end of the supply chain in supermarkets and 32% of samples were without detectable YM (Figure 2.5). The reduction in level and detection of YM at packing (95% of samples below detection) and no change through distribution (p<0.05) suggested the efficacy of washing, sanitizing, and handling practices to control YM and extend shelf life. The

maximum level of YM detected from the supermarket was 3.8 log CFU/g and along with the absence of visible spoilage of products prior to sale was consistent with the cut-off of at least 6-8 log CFU/g required for visible spoilage *(2).* Mold isolates visualized microscopically consisted of *Penicillium, Aspergillus, Alternaria, Fusarium, Geotrichum, Rhizopus* and *Trichoderma*.

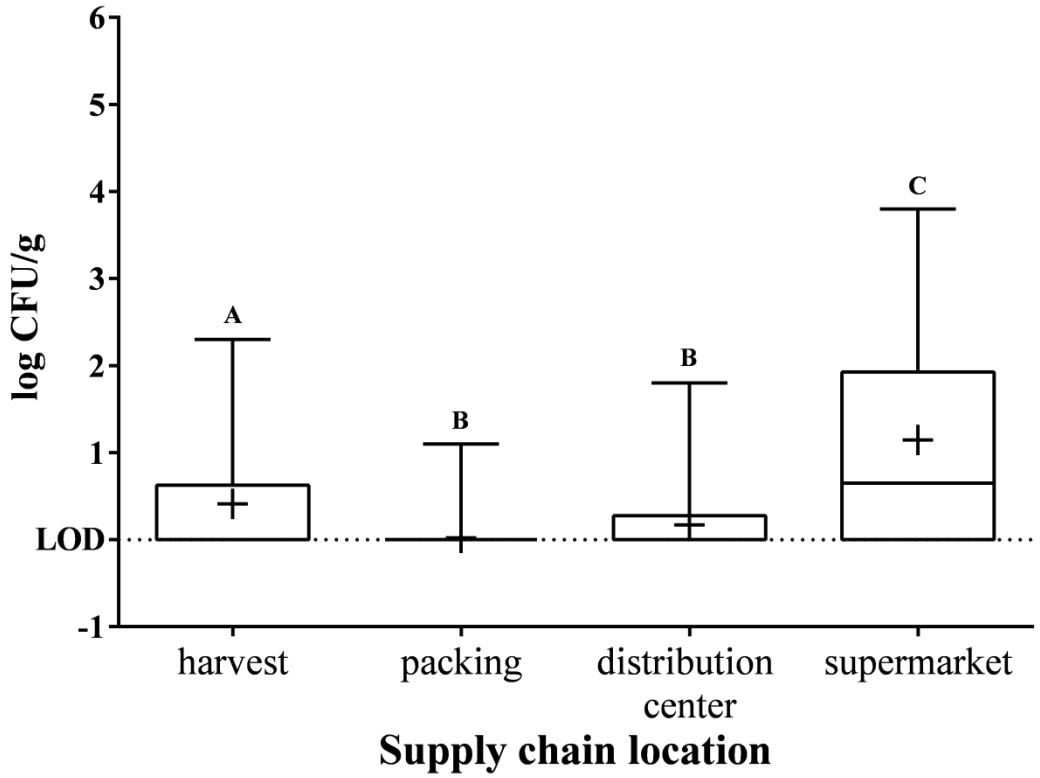

Figure 2.5 Yeasts and molds (log CFU/g) on tomatoes sampled from the postharvest supply chain. +: location geometric mean. LOD: limit of detection. A: boxes-and-whiskers marked with the same letter are not significantly different (Tukey-Kramer HSD, α=0.05).

2.3.5 Days postharvest at supermarket locations

For the sampling locations of harvest, packing and distribution, there was one unique location, while for the retail supermarket there were five unique locations sampled across the USA, due to the clientele and sales patterns of the distribution center. The length of the supply chain (number of days postharvest) differed for these supermarket locations. Table 2.1 shows the significant differences in the indicator populations as explained by the day the tomatoes were put on sale at the supermarket, suggesting that the increased levels may be attributed to "older" tomatoes. The cutoff for differences between days differed across indicators. The R^2 values were included to indicate the correlation between APC, TC and YM and days in the supply chain. Other factors not controlled in this study, but observed during sampling, that may further explain differences at the supermarket location could be storage and display temperatures or handling and hygiene practices.

Table 2.1 Indicator populations shown by the number of days postharvest prior to sampling at the supermarket location

Days postharvest at supermarket	**n=**	**APC** $R^2=0.75$[a]	**TC** $R^2=0.47$	**YM** $R^2=0.75$
6	10	1.3±0.6[b] A	0±0 A	0.3±0.3 A
7	16	2.4±1.1 A	0.5±0.9 A	0.2±0.5 A
8	17	4.1±0.7 B	1.0±1.0 AB	0.7±0.5 A
9	4	3.7±0.6 B	2.3±1.6 BC	0.1±0.3 A
10	24	4.6±0.5 B	2.6±1.4 C	2.7±0.9 B

[a] R^2 values for model of indicator population explained by day at supermarket
[b] Mean ± std deviation for log CFU/g aerobic plate count (APC), total coliforms (TC) and yeasts and molds (YM)
A: Means within an indicator population followed by the same letter are not significantly different (Tukey-Kramer HSD, α=0.05)

2.4 DISCUSSION

Notable outbreaks associated with fresh tomatoes in previous decades *(3, 4, 11, 13)* have warranted stricter practices and safety-focused marketing campaigns such that many tomato growers are already aware and compliant with modern food safety regulations. For example, the increasing use of protected agricultural systems, GAPs and traceability programs demonstrate actions to reduce foodborne illnesses associated with fresh tomatoes, both imported and domestic. Still, the unpredictable sources and mechanisms of contamination present research opportunities. Laboratory and field research have focused on contamination risks in certain locations of the supply chain, including the production environment and packinghouse design, as well as temperature control during transportation and retail *(15, 23, 29)*. Additional opportunities exist for development of more reliable and resilient supply chains for detecting and responding to contamination. In this study, three indicator microorganism populations, total aerobic mesophilic bacteria, total coliforms and yeasts and molds, were observed to have different changes on tomato surfaces as they were handled, packed and transported in an international supply chain from the field to the supermarket.

Overall, the microbial quality of tomatoes was very good, and the level of all indicators on tomatoes at harvest was several log CFU/g lower compared with published levels in tomatoes and other open-field fresh produce commodities *(5, 15)*. Initial APC coming from the field may consist of native microbiota on the plant or production environment, such as *Pseudomonas, Methylobacterium, Sphingomonas, Erwinia* and *Rhizobium (17, 19)*. In order to recover more detectable levels and

changes of indicators on tomatoes, total coliforms were chosen for quantification over fecal coliforms. The total coliform assay included detection of generic *E. coli* as an indicator of fecal contamination, however there was no detectable (<1 CFU/g) *E. coli* on tomatoes from this supply chain, which has also been reported in other produce supply chains *(12, 15, 23)*. The interpretation of total coliform results was made in consideration of the potential sources of coliforms (plant, soil, water, mammals). The level of TC at harvest was low and 85% of tomatoes had no detectable coliforms, which suggested the field and production environment were not sources of coliforms in this supply chain. Lastly, the YM population, along with APC, may allow for inference on fungal degradation of product quality in the supply chain. The initial level of YM at harvest was low and may have included common fungal pathogens of tomatoes such as *Alternaria alternaria, Phytophthora nicotiana* var. *parasítica*, *Botryotinia fuckeliana/Botrytis cinérea*, *Geotrichum candidum, Rhizopus stolonifer*, *Colletotrichum coccodes*, *Penicillium expansum*, and *Fusarium roseum*. Upon growth, these molds, and even yeasts, have proteolytic effects that can increase the pH of fruit and vegetable tissues to enable increased growth rates of surface bacteria *(24, 25)*.

The effect of the packinghouse practices differed between indicator populations and demonstrated that microbial levels can stay the same (APC), originate (TC) and decrease (YM) during washing, sanitizing, drying, sorting and packing. The APC population was not significantly different from harvest, the TC population significantly increased, and YM were significantly reduced and minimally detected at packing. Adding chlorine to the wash water is primarily intended to minimize the spread of contamination to other pieces of produce and surfaces during postharvest

handling, especially when using recirculated water. However in fresh produce applications, a properly functioning wash system should also reduce APC by 1-2 log to potentially increase shelf life and quality of products *(2)*. Here the use of 150 ppm total chlorine was monitored and maintained in the recycled water, and a 1 log CFU/g reduction of both APC, as shown by the control tomatoes sampled immediately after washing (Figure 2.2), and YM were achieved (Figure 2.5). Removing or "culling" poor quality tomatoes or those visibly infected with yeasts and molds was also implemented and may have contributed to the level of control. The increase in TC at packing aligns with recent hypotheses that introduction of contamination occurs during packing and handling steps. The magnitude of increase in TC between harvest and packing was similar to levels reported for parsley, cilantro, mustard greens and cantaloupe *(15)* as well as for tomatoes *(23)* and suggested the postharvest source of coliforms on tomatoes may be processing water, workers, or food contact surfaces in the packinghouse.

The increases in APC, TC and YM through the distribution and supermarket locations suggested the effects of more variable temperature, time and microbial conditions in those storage and handling environments, when compared to control tomatoes. On control tomatoes in 10 days of storage at 10°C and 90% RH, APC did not show significant increase above 2 log CFU/g and TC and YM remained undetected. The increase in APC observed between packing, distribution and supermarket in this supply chain study was more similar to literature values of tomatoes stored at 21°C for ten days *(5)*. While the TC population declined at the distribution center storage, there was a significant increase in level and variation in

supermarkets, indicating that microorganisms were originating or increasing due to handling and storage conditions. There are no studies that report indicators on tomato surfaces sampled from retail supermarkets in the USA, but one study from Mexico showed similar APC and TC levels in local and retail market tomatoes to be 3.2-3.6 log CFU/g and 2.6 log CFU/g, respectively *(10)*. As the maximum values of APC and YM on tomatoes in the studied supply chain were <6 log CFU/g and there was no observed spoilage, it was concluded that postharvest control for quality was effectively implemented to ensure shelf life of the tomatoes was well over ten days. In comparing the indicator levels at the supermarket location across days postharvest, the results showed that the increased levels may be explained by length of supply chain, but that there may also be other variables contributing to supermarket differences that warrant further study and intervention.

This pilot study demonstrated microbial dynamics as a proof-of-concept that supply chain locations and practices influence microbial populations on fresh tomato surfaces. Quantification of these indicator microorganism populations on tomato surfaces also demonstrated the level of control that can be achieved in a well-maintained system. It is recognized that these microbial populations on produce are not necessarily indicators of food safety risk or pathogen prevalence, but are useful in identification of areas of production, transportation and sale of fresh produce that may be susceptible to introduction microorganisms and/or have conditions conducive for their proliferation. The results suggest that growers and produce handlers review packing and supermarket locations as crucial points to control microbial quality, especially considering the potential behavior if pathogenic microorganisms were

introduced to a fresh produce supply chain. The large variances observed in some locations indicate opportunities for improved sampling, study and handling practices. Future studies may include more lots, several farms and distribution centers, multiple harvest years, both protected agricultural system and open field surveys, temperature monitoring, and domestic and imported products. Although this was one supply chain of one fresh produce commodity during one harvest season, the results may inform future survey studies and/or facilitate research, practices, and risk simulations to prevent product contamination and outbreaks of foodborne illness.

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CHAPTER 3

RISK FACTORS FOR PREVALENCE AND CONCENTRATION OF INDICATOR MICROORGANISMS ON FRESH TOMATOES IN THE POSTHARVEST SUPPLY CHAIN

ABSTRACT

"Prevalence" and "concentration" describe the distribution of microbial populations in fresh produce units, capturing rare but significant occurrence of contamination. While estimates for human pathogens are difficult to obtain experimentally or by regular surveillance, indicator microorganisms can demonstrate similar potential behavior. In this study, the use of quality and safety microbial indicators on fresh tomatoes provided a framework to quantify the influence of spatial and temporal factors of the postharvest supply chain on prevalence and concentration. Microbial count data (log CFU/g) of aerobic plate count (APC), total coliforms (TC) and yeasts/molds (YM) on the surface of Roma tomatoes sampled from lots moving through a supply chain were used in mixed linear and logistic regression models to determine significant factors for concentration and prevalence, respectively. Location explained prevalence changes in TC (p<0.05) and YM (p<0.05), while days-in-transit best explained concentration dynamics in all populations (p<0.05), with each additional day contributing 0.5 log on average. Used together, these models quantified the dynamics observed (% prevalence, LS mean±s.e.). For example, at harvest TC had low prevalence in sampled tomatoes (13%), but high concentrations (2.7±0.5 log). After packing, TC prevalence (53%) and concentration (3.1±0.4 log) increased, while at the distribution center both decreased (30%, 0.6±0.2 log). At supermarkets, prevalence increased (55%) while concentration was variable (0.3-4.2 log/tomato).

Overall, locations with increased prevalence and variability were packinghouse and retail and the difference in concentration between a six- and ten-day supply chain was 2 log CFU/g. These results can be used in future risk assessment models.

Keywords: fresh produce, safety, postharvest, prevalence

3.1 INTRODUCTION

Outbreaks of *Salmonella* in tomatoes in 1990 and 1993 were likely contaminated in the packing shed, where a common dump tank was used for washing field-grown tomatoes in chlorinated water *(10)*. A multi-state outbreak of *Salmonella* in 2001 was associated with raw tomatoes sourced from a grower/packer operation washing, waxing, packing and shipping tomatoes to dicing processor, restaurants, and nursing homes *(11)*. Again between 2004 and 2006, there were annually recurring multi-state outbreaks of *Salmonella* associated with raw tomatoes with the supposed sources being either irrigation pond water, suspected animal infiltration or unidentifiable, respectively *(14)*. These scenarios are only a select few to represent the microbial and logistic dynamics that culminate in large geographically dispersed outbreaks presumed to be caused by sporadic or low-level contamination of widely distributed food items.

Furthermore, investigations often fall short in identifying the source of contamination due to many reasons: short growing season, lack of labeling, consumption with other foods, fast distribution and consumption of products with short shelf-lives, and the inherently untraceable network of fresh produce supply chains. For example, the large outbreak in 2008 of *Salmonella* Saintpaul was originally attributed to raw imported tomatoes prior to the investigations which linked the microbiologic evidence to jalapeño and serrano peppers *(2)*. While advancement of rapid detection methods is helpful to investigators, more research is also needed to understand the influences of the supply chain on the risk of microbial contamination as well as its subsequent spread or behavior due to the multiple handling steps prior to

consumption. A previous review of data from past outbreaks associated with raw tomatoes reported to the Centers for Disease Control and Prevention (CDC) reinforced this stating that most outbreaks associated with tomatoes were consumed at restaurants, but that traceback investigations suggested contamination occurred at farms, packinghouses or fresh-cut processing facilities *(3)*. Therefore, the effect of a single point of contamination can carry large consequences when the product volume or distribution time increase or when quality of the handling environment is poor.

In microbial risk modeling of food, the terms "prevalence" and "concentration" have been used to describe the spatial distribution of microorganisms in product units within lots, capturing the often rare but significant occurrence of contamination. Prevalence indicates the percentage of units with the presence of microorganisms and is usually determined by qualitative detection methods, while concentration uses quantitative or semi-quantitative enumeration to estimate the number of microorganisms in the sampled unit, usually expressed as the logarithmically transformed cell count per gram of product (log CFU/g). Used together, these characteristics of a batch of food product can convey either a systemic contamination point (high prevalence and low level concentration) or a point-source contamination (low prevalence and high level concentration), for example. Danyluk and Schaffner *(5)* in their risk assessment for *E. coli* in leafy greens define the three critical variables for estimating risk of product contamination in the field as the pathogen level [concentration], the number of days the produce is in the field prior to harvest, and the fraction of produce from the field that is actually contaminated [prevalence]. They then included subsequent models to explain how the practices and conditions to which

the spinach is exposed from the field to packaging to consumption alter the original contamination level, finalizing the exposure assessment.

The most common limitations to conducting accurate estimates of exposure to contamination are the abundance of zero counts from product and environmental sampling data *(6)* and the definition of the outcome (such as concentration and bacterial count). The zero values from the enumeration data can be interpreted as artificial zeros, due to chance or sample size, as true zeros or as censored below the level of detection. According to Duarte, et al. *(7)*, censoring zeros may not always be appropriate and threshold values should be excluded from analysis of microbial data. Furthermore, the authors argue that characterization of the microbial contamination should be made in a single enumeration step to prevent prediction of highly improbable outcomes. Pouillot et al. *(13)*, in their study on the impact of modeling concentration or bacterial number on risk estimates, found that modeling concentrations tends to overestimate risk in some scenarios by >10-fold as opposed to bacterial numbers. They suggest alternative approaches such as modeling the number of bacteria in contaminated units or the expected number of bacteria in positive units.

In an attempt to study this phenomenon in a fresh produce supply chain, a previous study has shown that indicator microbial populations of quality and safety do in fact change significantly on tomato surfaces during postharvest handling events and transportation (Chapter 2). Here the main research goal was to estimate how microbial surface indicator populations spread through a lot of produce given the environmental and logistic conditions between the field and supermarket by analyzing changes in prevalence and concentration. Microbial indicators of quality and safety—

aerobic plate count, total coliforms, and yeasts and molds—on fresh tomatoes were used to determine and estimate the significant factors of the postharvest supply chain that influence these dynamics. Although these are very loosely linked to product contamination with human pathogens, their common and more detectable presence are what make them useful for studying the concept of microbial dynamic trends within a postharvest supply chain of fresh produce. In addressing the previously mentioned limitations, microbial data were obtained completely from enumeration methods. Using a hurdle model, count data from resulting contaminated and uncontaminated tomatoes were analyzed together to determine prevalence and from contaminated units to determine the expected log number of bacteria in positive units.

3.2 DATA

3.2.1 Supply chain factors

Roma tomatoes were produced on a farm located in the state of Nuevo León, México utilizing protected agriculture systems of greenhouses and shade houses, which corresponded to different lot codes of the final products. Tomatoes were hand-harvested into plastic containers and transported to a packinghouse located within the same farm, where they were washed in chlorinated water, conveyed through a sorting line and hand-packed into boxes. Palletized boxes were loaded onto refrigerated (10-12°C) trailer trucks for transportation to and storage in a distribution center in southern Texas, USA. Tomato boxes were sold based on size and color to retail supermarkets across the USA.

The spatial and temporal factors to study from this supply chain included: sampling location in the supply chain, harvest date during the production year, and

number of days the tomatoes were in transit from field to supermarket. The sampling location (further referred to as "location") encompassed the handling practices and environmental conditions at significant points along the supply chain: harvest, packing, distribution center, and retail. The harvest date (further referred to as "time of harvest") captured the seasonal temporal effect, lot to lot, during the production year in northeastern Mexico (May-November) and was measured by sampling tomatoes over five months (June-October). The number of days-in-transit (further referred to as "days") tracked the day-to-day temporal effect within each lot moving through the supply chain. For example, tomatoes were always harvested and packed on the same day, arrived to the distribution center the next day, were sampled from the distribution center between four and five days postharvest, and were sampled from supermarkets across the US between six and ten days postharvest.

3.2.2 Sample collection

In all instances, the same lot codes designated by the farm and displayed on each box were followed through the supply chain for a total of 28 different lots. The first sampling location was upon arrival to the packinghouse after harvesting, where tomato samples were taken with gloved hands from their plastic harvesting containers. The second sampling location was after the washing and sorting steps and immediately prior to boxing, where tomato samples were taken by the workers. The third sampling location was with gloved hands from the palletized boxes in the storage room of the distribution center, after several days of storage and prior to shipment. The final sampling location was with gloved hands from the point of sale in supermarkets or in a few occasions from the supermarket storage room. Four to ten

tomato samples were taken individually in Ziploc bags from boxes of each lot code, depending on the sampling location, for microbial analysis. A total of 475 tomatoes were collected and analyzed at Cornell University, Ithaca, NY USA or the Autonomous University of Nuevo Leon, San Nicolas, NL Mexico.

3.2.3 Microbial data

Microbiological analyses were conducted on individual tomatoes for the following indicator populations of interest to the models: aerobic plate count (APC), total coliforms (TC), and total yeasts and molds (YM). Microbial count data are presented in logarithm base ten of colony forming units per tomato (log_{10}CFU/g), with the limit of detection of analysis being 1 CFU/g (or 0 log CFU/g). The materials and methods and descriptive analysis of these results have been previously reported (in Chapter 2).

3.3 STATISTICAL MODELS

Beyond observing that the selected microbial indicator populations change overall on tomatoes in the course of the supply chain (Chapter 2), analyzing the data with two time frames (consecutive days and calendar time of harvest) in addition to the location, prompted two subsequent points of interest: (1) how exactly did the indicator populations change over time and/or space and (2) which factors of the supply chain (time and/or space) were associated with this dynamic behavior. Furthermore, the overall population changes were different across the three indicator microorganisms, suggesting that the risk factors influencing their changes may also be different. This information may be useful for guiding preventive practices against and detection of undesirable microbial populations on fresh produce.

In approaching these research questions, given the response (log CFU/g) and indicator variables (location, days, time of harvest), linear regression was an appropriate model to select for analyzing the concentration. Because the tomato samples originated from designated "lots" of product at each time of harvest, the samples within the same lot were correlated and the effect of lot was nested within time of harvest. Furthermore, the lots sampled were not representative of all tomato lots exported to the USA and its contribution to model error was not of interest. Therefore, the model was designed to be a mixed linear model with lot, nested within time of harvest, as a random effect.

However, preliminary examination of the dataset showed very different dynamics and distributions between populations that did not satisfy the model assumptions. The distribution of APC on tomato samples was normal, while a zero-inflated distribution for samples analyzed for total coliforms and yeasts and molds on tomatoes was observed (Figure 3.1). This presented a common phenomenon in environmental microbial surveillance and the decision regarding how to interpret such "zeros" in further analysis. Because the microbiological analysis was sensitive and made per tomato (detection limit: 1 CFU/g or 0 log CFU/g) and analysis of within lot variation at each location was not significant, zeros were treated as true zeros and the data was split accordingly to better understand this phenomenon as prevalence, specifically on tomatoes moving though a supply chain, and to satisfy model assumptions. Therefore, in this analysis, a hurdle model was employed to characterize the change within populations in terms of prevalence and concentration.

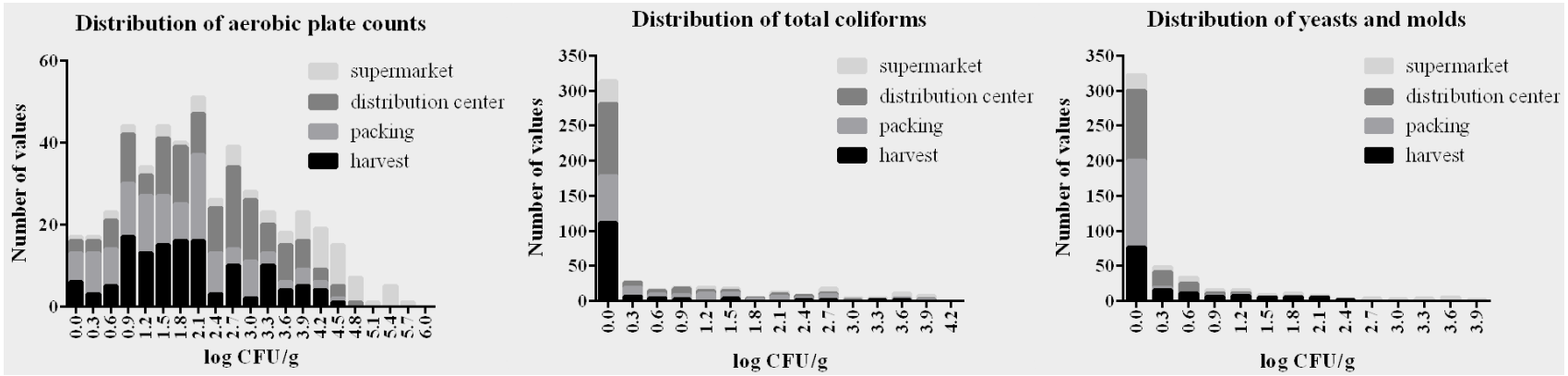


Figure 3.1 Distribution of indicator populations on tomatoes sampled from different locations along the supply chain

3.3.1 Logistic regression model for indicator prevalence

Because APC captures an abundant microbial population, the data were not zero-inflated and the concept of prevalence was constant, near one. However, this was not the case for the less prevalent TC and YM populations. Therefore, based on the limit of detection, count data for TC and YM were converted into a binomial variable for presence and modeled using a Generalized Estimating Equation with a binomial distribution and logit link function (PROC GENMOD, SAS 9.3) to estimate the conditional probability of TC and YM on tomatoes, further referred to as prevalence. Indicator variables were included for location in the supply chain, time of harvest and days in the supply chain. Because multiple tomato samples were taken from the same lot at different locations in the supply chain over different harvest dates, lot number was nested within harvest and was treated as a repeated subject with a compound symmetry correlation structure to account for possible correlation between tomato samples. The model predicted natural log odds of tomatoes with detectable presence of TC or YM, conditional upon significant indicator variables:

$$ln(\frac{p}{1-p}) = \alpha + \beta_i(location) + \beta_j(harvest) + \beta_k(days) + lot[harvest] + \varepsilon$$

where p is the probability of presence and $\frac{p}{1-p}$ is the probability of presence over the probability of absence, or odds. The main categorical effects (location and time of harvest) were estimated in terms of β_i and β_j, for each level i or j, respectively. The continuous effect of days was estimated in terms of β_k, the incremental log odds for each consecutive day k in the supply chain. Lot, nested within harvest, was included in the model as a random effect and was therefore not estimated.

LS means are predicted population margins, that is, they estimate the marginal means over a balanced population (linear combination of parameter estimates corresponding to the level of the class variable). Here they are presented as the predicted population margins of the log odds for each location in the supply chain over the other variable, time of harvest. With the logit link function, they can be transformed to the data scale to be interpreted as the probability of presence of TC or YM at each location. Lastly, from the expected odds, comparisons can be made across locations, known as odds ratios and interpreted as higher (>1), equal (=1) or lower (<1) odds of the present population.

3.3.2 Mixed linear regression model for indicator concentration

Microbial count data (log CFU/g) for APC, TC and YM on the surface of Roma tomatoes sampled within lots moving through a supply chain were used in mixed linear models (PROC MIXED, SAS 9.3) to determine significant factors for the level of concentration. Fixed effects were included for location in the supply chain, time of harvest and days in the supply chain, and lot was a random effect nested within harvest to demonstrate the shared compound symmetry covariance between tomatoes sampled from the same lot. The model predicted the concentration of indicator microorganism populations on tomato according to significant main effects:

$$y = \alpha + \beta_i(location) + \beta_j(harvest) + \beta_k(days) + lot[harvest] + \varepsilon$$

where y is the concentration, log CFU/g. The main categorical effects (location and time of harvest) were estimated in terms of β_i and β_j, for each level i or j, respectively. The continuous effect of days was estimated in terms of β_k, the increment to concentration for each consecutive day k in the supply chain. Lot, nested

within harvest, was included in the model as a random effect and was therefore not estimated.

The models were first run using the whole data set for APC, TC and YM, but due to the high frequency of undetectable levels of TC and YM, those data did not meet the model assumptions, as previously mentioned. Instead, those data were removed of zeros and linear models for these populations were run using only the data from tomatoes with detectable TC and YM. The models were also run excluding the temporal factor, days, to demonstrate the impact of considering both spatial and temporal factors of the supply chain. No interaction terms were considered. AIC values were used to compare models and determine the best fit.

3.4 RESULTS

3.4.1 Risk factors for microbial indicator prevalence

3.4.1.1 Total Coliforms

Location in the supply chain and time of harvest were the spatial and temporal factors associated with changes in prevalence of TC (Table 3.1). Number of days in transit through the supply chain did not explain the changing prevalence. While the source of coliforms could be environmental (plant, soil, water) or due to poor sanitary practices (food-contact surfaces, workers), the results of the logistic regression model suggested that the increased presence was due to postharvest handling in packinghouse and supermarket locations. The intercept explained the log odds of TC at harvest in June and was -1.0. This is interpreted as a low expected odds (<1) and that the probability of absence at harvest for the first time of harvest is higher than the probability of presence. Furthermore, there were significantly higher increments to

the log odds of TC on tomatoes at the packinghouse (2.1±0.3) and supermarket (2.1±0.4), but not in the distribution center where tomatoes were stored in pallets until sold.

The times of harvest towards the end of the growing season, late August through October, had significant effects on the log odds of TC on tomatoes and were reducing effects. The other temporal factor, days in the supply chain, was not significant for inclusion in the model, suggesting that changes in prevalence of TC are driven more by spatial conditions verses seasonal or logistics factors.

Table 3.1 Logistic regression model of presence *(y=1)* of TC on tomatoes

	Parameter Estimate (β)	Standard Error	*95% CI*
Intercept (α)	-1.0	0.3	(-1.5, -0.5)
Location			
harvest	0.00	----	----
packinghouse	2.1	0.3	(1.4, 2.7)
distribution center	1.0	0.4	(0.3, 1.8)
supermarket	2.1	0.4	(1.4, 2.9)
Time of Harvest			
June	0.00	----	----
early July	-0.9	0.4	(-1.8, -0.0)
mid-July	0.3	0.3	(-0.2, 0.7)
early August	-0.1	0.1	(-0.3, 0.1)
late August	-2.2	0.4	(-3.1, -1.4)
September	-1.7	0.2	(-2.1, -1.2)
October	-2.5	0.8	(-4.0, -1.0)

Further analyses of the location effect using probability and odds ratio (OR) for each location demonstrated the changing prevalence of TC on tomatoes due to location differences across the supply chain (Table 3.2). The prevalence of TC increased from 11% in the harvested tomatoes to 50% of the washed and packed tomatoes. The prevalence of TC decreased to 27% during distribution and storage, but

then increased back to 52% in supermarkets. The significant increases in prevalence compared with that of tomatoes coming from the field were at the packinghouse and supermarket with 7.8 and 8.5 times more likely to find tomatoes with TC, respectively.

Table 3.2 Location LS Means and Odds Ratios of TC prevalence

Location	LS Means Estimate (β)	Standard Error	*95% CI* for estimate	Expected odds (e^{β})	Probability, y=1 ($p = \frac{e^{\beta}}{1+e^{\beta}}$)	*95% CI* for Probability	Odds Ratio	*95% CI* for Odds Ratio
harvest	-2.1	0.3	(-2.7, -1.4)	0.1	0.11	(0.1, 0.2)	base	----
packinghouse	0.0	0.2	(-0.4, 0.4)	1	0.50	(0.4, 0.6)	7.8	(3.3, 19)
distribution center	-1.0	0.2	(-1.5, -0.6)	0.4	0.27	(0.2, 0.4)	2.8	(1.0, 7.9)
supermarket	0.1	0.3	(-0.4, 0.6)	1.1	0.52	(0.4, 0.6)	8.5	(3.1, 24)

3.4.1.2 Total yeasts and molds

Location was the only significant factor to explain prevalence changes of YM on tomatoes moving from field to retail, while temporal factors had no effect. Therefore, the model parameter estimates and the reported LS Means provide the same information (Table 3.3). The prevalence of YM at harvest was 43% of tomatoes. This decreased after washing to 5%, indicating an effective level of chlorine in the water and proper culling practices to remove visually damaged or rotting tomatoes. Prevalence of YM during distribution and storage increased to 31%, perhaps due to circulating air in trailer trucks or warehouse rooms. Finally, prevalence of YM reached its maximum in supermarkets with 71% of the sampled tomatoes containing detectable levels. The differences between locations are magnanimous. Compared to the packed product, tomatoes at distribution and retail supermarkets were 9.0 and 51 times more likely to contain YM, respectively.

Table 3.3 Location LS Means and Odds Ratios of YM prevalence

Location	LS Means Estimate (β)	Standard Error	*95% CI* for estimate	Expected odds (e^{β})	Probability, y=1 ($p = \frac{e^{\beta}}{1+e^{\beta}}$)	*95% CI* for probability	Odds Ratio	*95% CI* for Odds Ratio
harvest	-0.3	0.2	(-0.8, 0.1)	0.7	0.43	(0.3, 0.5)	15	(5.0, 44)
packinghouse	-3.0	0.5	(-3.9, -2.1)	0.05	0.05	(0.0, 0.1)	base	----
distribution center	-0.8	0.2	(-1.2, -0.4)	0.4	0.31	(0.2, 0.4)	9	(2.6, 31)
supermarket	0.9	0.5	(-0.1, 1.9)	2.5	0.71	(0.5, 0.9)	51	(6.4, 410)

3.4.2 Risk factors for microbial indicator concentration

3.4.2.1 Aerobic plate count

Location, time of harvest and days were all significant (p<0.05) factors for APC concentration (Table 3.4). Contrary to prior hypotheses, results from the linear regression of APC showed that the high concentrations in the supermarket, at the end of the supply chain, may not have been due to practices or conditions at that location (β_4=-1.6), but instead to the fact that the tomatoes were at their oldest (6-10 days) and each day contributed 0.4±0.1 log CFU/g. The intercept was 2.1±0.3 log CFU/g, indicating the amount on tomatoes at harvest in the beginning of the season. The washing and packing processes decreased the APC population by 0.2±0.1 log. Distribution and storage under controlled atmosphere decreased the APC population by 1.5±0.4 log. Finally, storage and sale in the retail supermarket decreased the APC population by 1.6±0.6 log, relative to the intercept.

Including days in the model adjusted the effect of location, as can be seen when comparing columns (a) and (d). When days was excluded from the model (in Chapter 2), the intercept was greater and the effect of location was increasing as the

tomatoes moved from farm to retail, with the exception of packinghouse, which always occurred on the first day. Excluding days from the model also reduced the standard error of the location effects, columns (b) and (e), masking the variability observed within each location.

There was a similar trend for time of harvest as that for prevalence, which was increasing reductions in concentration towards the end of the harvest season. The change to the harvest effect when days is included is only in magnitude, not direction, which was expected.

Table 3.4 Mixed linear regression model of APC concentration on tomatoes

	Parameter Estimate (β) (a)	Standard Error (b)	*p*-Value (c)	Parameter Estimate (β) (d)	Standard Error (e)	*p*-Value (f)
Intercept (α)	2.1	0.3	<0.05	2.6	0.3	<0.05
Location						
harvest	0.00	----	----	----	----	----
packinghouse	-0.2	0.1	>0.05	-0.2	0.1	>0.05
distribution center	-1.5	0.4	<0.05	0.4	0.1	<0.05
supermarket	-1.6	0.6	<0.05	1.5	0.2	<0.05
Time of Harvest						
June	0.00	----	----	----	-----	----
early July	0.2	0.3	>0.05	-0.1	0.3	>0.05
mid-July	-0.3	0.3	>0.05	-0.3	0.3	>0.05
early August	-0.4	0.2	>0.05	-0.3	0.3	>0.05
late August	-1.0	0.3	<0.05	-1.0	0.3	<0.05
September	-1.0	0.3	<0.05	-1.3	0.3	<0.05
October	-1.0	0.3	<0.05	-1.3	0.3	<0.05
Days	0.4	0.1	<0.05	excluded	----	----
Model AIC (small)		1357.7			1378.1	

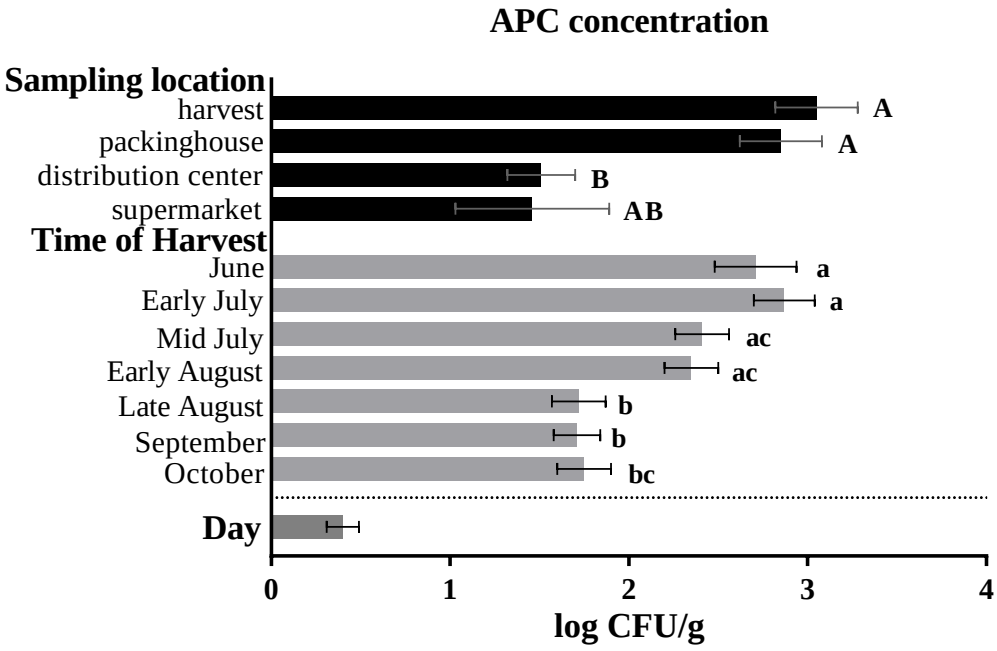


Figure 3.2 LS means of significant factors associated with changes in APC concentration on tomatoes in the supply chain. A/a: bars marked with the same letter are not significantly different (Tukey-adjusted *p*-values, α=0.05).

3.4.2.2 Total coliforms

Location, time of harvest and days were all significant factors for TC concentration (Table 3.5). For TC, there is an unexpected change in location trends compared to APC, but no significant differences between times of harvest. Additionally, it was observed that the main effect of days explains most of the increasing concentration of TC on tomatoes moving through the supply chain, contributing 0.5±0.1 log/g per additional day. The intercept was 0.6±0.3 log CFU/g, representing the lower concentration of TC on tomatoes coming from the field at harvest. In this population, the washing and packing steps increased the concentration by 0.3±0.2 log, but was not significant (p>0.05). Distribution and storage under controlled atmosphere decreased the population by 2.0±0.7 log. Finally, storage and

sale in the retail supermarket decreased the population by 2.2±1.2 log.

Again, including days in the model adjusted the effect of location, as can be seen when comparing columns (a) and (d). When the variable days was excluded from the model, the intercept was greater and the effect of location was increasing as the tomatoes moved from farm to retail. The effects at the packinghouse and the distribution center were not significant (p>0.05). Excluding days from the model also reduced the standard error of the location effects, columns (b) and (e), masking the wide variability observed within each location.

Time of harvest was a significant main effect in the model (p<0.05), but none of the individual times of harvest were significantly different from zero or one another. This was not altered when days was removed from the model. The only time of harvest that did not reduce the TC population was mid-July (0.4±0.3, p>0.05).

Table 3.5 Mixed linear regression model of TC concentration on tomatoes

	Parameter Estimate (β) (a)	Standard Error (b)	*p*-Value (c)	Parameter Estimate (β) (d)	Standard Error (e)	*p*-Value (f)
Intercept (α)	0.6	0.3	>0.05	1.2	0.3	<0.05
Location						
harvest	0.00	----	----	0.00	----	----
packinghouse	0.3	0.2	>0.05	0.3	0.2	>0.05
distribution center	-2.0	0.7	<0.05	0.1	0.3	>0.05
supermarket	-2.2	1.2	>0.05	1.4	0.3	<0.05
Time of Harvest						
June	0.00	----	----	0.00	----	----
early July	-0.2	0.3	>0.05	-0.6	0.3	<0.05
mid-July	0.4	0.3	>0.05	0.4	0.3	>0.05
early August	0.0	0.3	>0.05	0.2	0.3	>0.05
late August	-0.1	0.4	>0.05	-0.1	0.4	>0.05
September	-0.1	0.3	>0.05	-0.5	0.3	>0.05
October	-0.5	0.4	>0.05	-0.7	0.4	>0.05
Days	0.5	0.1	<0.01	excluded	----	----
Model AIC (small)		438.4			446.6	

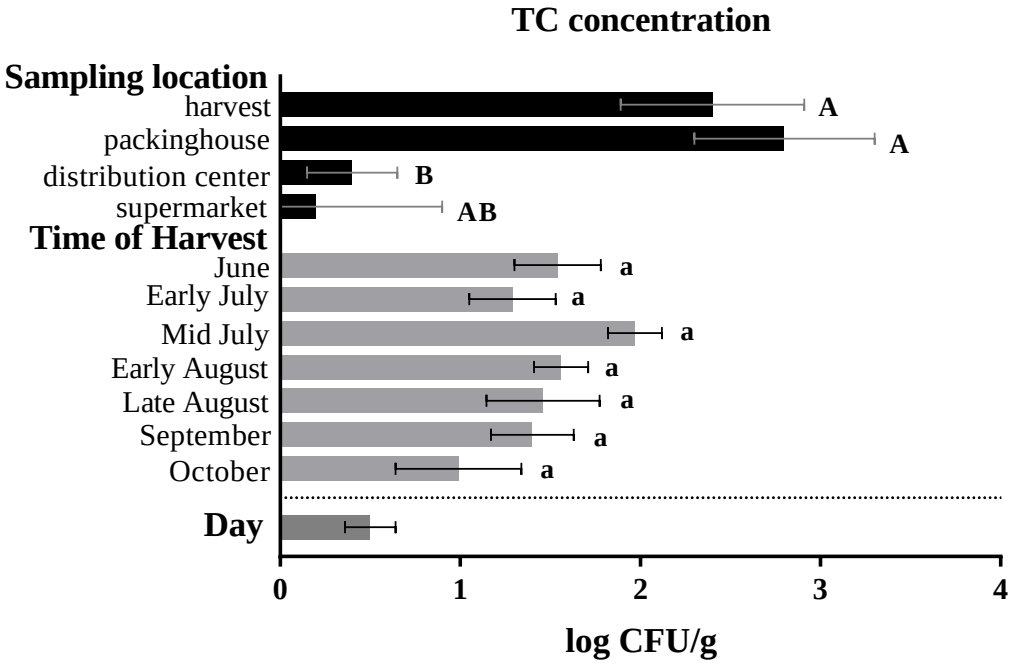


Figure 3.3 LS means of significant factors associated with changes in TC concentration on tomatoes in the supply chain. A/a: bars marked with the same letter are not significantly different (Tukey-adjusted *p*-values, α=0.05).

3.4.2.3 Total yeasts and molds

For YM, the seasonal factor time of harvest, did not explain changes in concentration, only location and days were significant factors (Table 3.6). The intercept was 0.5±0.1 log CFU/g, representing the lower concentration of YM on tomatoes coming from the field at harvest. From there, the washing and packing processes decreased the population by 0.5±0.3 log. Distribution and storage under controlled atmosphere decreased the population by 2.8±0.3 log. Finally, storage and sale in the retail supermarket decreased the population by 3.3±0.5 log. Interestingly, again each day in the supply chain contributed 0.5±0.1 log.

In this population, including days in the model adjusted the effect of location, but only at the supermarket, as can be seen when comparing columns (a) and (d).

When days was excluded, the decreases after washing and packing and during distribution changed slightly in magnitude but not in significance. Excluding days increased the impacts of the intercept and the supermarket location, 1.0±0.1 and 0.6±0.2 log, respectively, capturing the highest levels of YM concentration observed at harvest and supermarkets.

Table 3.6 Mixed linear regression model of YM concentration on tomatoes

	Parameter Estimate (β) (a)	Standard Error (b)	*p*-Value (c)	Parameter Estimate (β) (d)	Standard Error (e)	*p*-Value (f)
Intercept (α)	0.5	0.1	<0.05	1.0	0.1	<0.05
Location						
harvest	0.00	----	----	0.00	----	----
packinghouse	-0.5	0.3	<0.05	-0.4	0.3	>0.05
distribution center	-2.8	0.3	<0.05	-0.5	0.1	<0.05
supermarket	-3.3	0.5	<0.05	0.6	0.2	<0.05
Days	0.5	0.1	<0.05	excluded	----	----
Model AIC (small)		311.1			345.3	

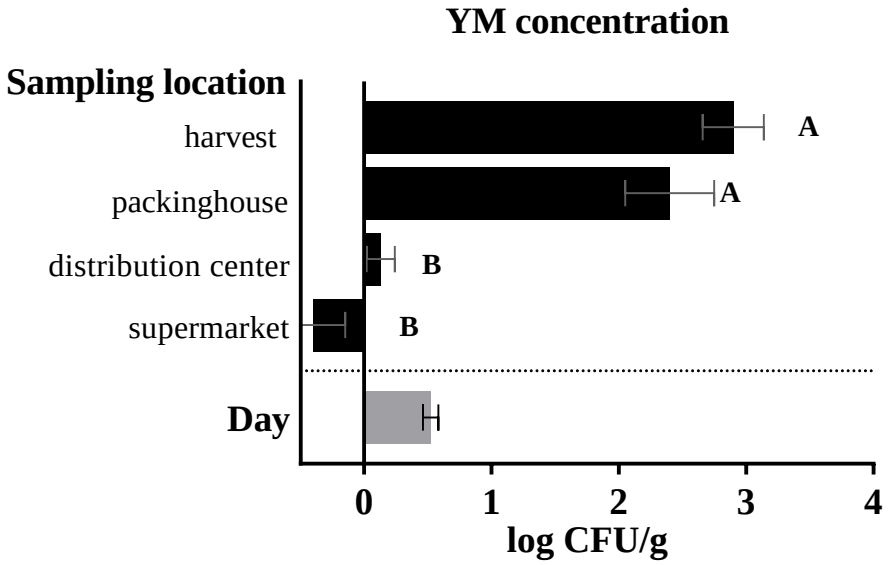


Figure 3.4 LS means of significant factors associated with changes in YM concentration on tomatoes in the supply chain. A: bars marked with the same letter are not significantly different (Tukey-adjusted *p*-values, α=0.05).

3.4.3 Use of prevalence and concentration models for postharvest supply chain exposure assessment and intervention

The significance of these two models, prevalence and concentration, for assessing the resiliency of the supply chain to potential contamination events is when they are used in combination. Predicting how microbial populations increase in the number of products within a lot and/or in levels on a single product illustrates how sporadic or low-level contamination may evolve into wider dispersed public health risks due to postharvest conditions. Alternatively, these models can demonstrate how some supply chains may effectively reduce a contamination event to an acceptable level of risk.

The model parameters in Sections 4 and 5 were used to predict levels of exposure in tomatoes for different supply chain scenarios (Figure 3.5). For example, controlling total coliforms, which had low prevalence and lower concentration in sampled tomatoes at harvest (11%, 1.1 log CFU/g) and increased after packing (50%, 1.4 log), may require revisiting the food safety hazards or focusing resources in areas in packinghouses that spread or support contamination instead of the production environment. During distribution, both TC prevalence and concentration decreased (27%, 0.6 log), but the supermarket location increased prevalence and concentration, differing depending on the length of time prior to sale (1.4-3.4 log CFU/g, 6-10 days respectively).

On the control of quality and spoilage microorganisms, the product cooling, chlorine wash and packinghouse operations reduced both YM prevalence and concentration (5%, 0.5 log CFU/g). Circulated air during distribution and storage may

have contributed to the spread of YM within the lot of tomatoes in the distribution center, but effectively diluted the concentration to below detectable levels, as prevalence increased and concentration per tomato decreased (31%, -0.3log CFU/g). However, this population both spread and increased concentration in the time to sale and at supermarket conditions (71%, 0.2-2.2 log CFU/g). As some spoilage bacteria and fungi can decay plant tissue at temperatures below 4°C, it is expected that the highest counts occur at the end of the supply chain in the supermarket *(1)*. Additionally, as fresh tomatoes were often incorporated into existing bins of produce on sale, the highest prevalence across the three indicators occurred at the supermarket.

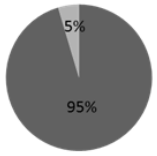
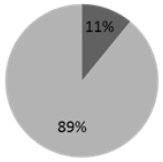
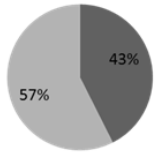
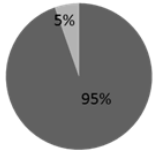
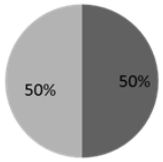
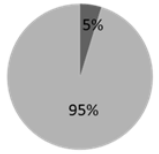
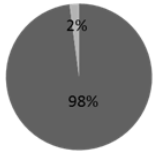
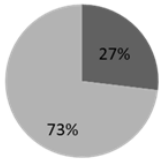
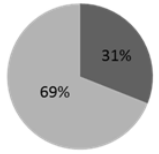
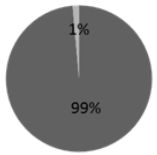
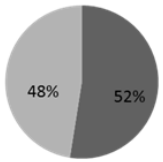
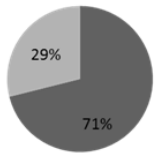
Supply Chain Location	Aerobic Plate Count		Total Coliforms		Yeasts and Molds	
	Prevalence (■ =present, ■ =absent)	Concentration (log CFU/g)	Prevalence (■ =present, ■ =absent)	Concentration (log CFU/g)	Prevalence (■ =present, ■ =absent)	Concentration (log CFU/g)
Harvest (day=1)		**2.5**		**1.1**		**1.0**
Packing (day=1)		**2.3**		**1.4**		**0.5**
Distribution Center (day=4)		**3.5**		**0.6**		**-0.3**
Supermarket (day=6-10)		6 days: **2.9** 10 days: **4.5**		6 days: **1.4** 10 days: **3.4**		6 days: **0.2** 10 days: **2.2**

Figure 3.5 Predicted prevalence and concentration of the indicator populations on tomatoes at each location in the supply chain

3.5 DISCUSSION

3.5.1 Prevalence

Most published literature on prevalence and fresh produce, report the low prevalence or absence of foodborne pathogens, such as *Salmonella* and *E. coli* O157:H7, on fruits and vegetables or environmental samples along the production chain *(4, 6, 8, 9, 12, 15)*. Many of these same reports fail to find prevalence of pathogens or even generic *E. coli* on produce samples. Therefore, the conclusions made on the global trends of contamination prevalence along the supply chain of fresh produce are limited the field and packing locations as risk areas for contamination. Furthermore, few studies report the impact of pre- and postharvest practices on spoilage microorganisms of interest for the retail produce industry.

In this study, the changes in prevalence for total coliforms and yeasts and molds presented were associated more with spatial factors of the tomato supply chain. In both TC and YM populations, the prevalence was most influenced by the location effect, not the time of harvest or number of days in the supply chain, which suggested that the practices or conditions at each location vary in how they either spread or controlled microbial populations on tomatoes within a lot. Furthermore, the effect of moving sequentially along the supply chain on prevalence was not consistently increasing or decreasing, but dynamically changing, nor was it consistent between the microbial populations. These dynamics may be supported by the different microbial physiology between bacteria and yeasts and molds. While most microorganisms on whole fruit or vegetable surfaces in the field are soil inhabitants and can be transferred via soil particles, airborne spores and irrigation water *(1)*, yeast and mold prevalence on

sampled tomatoes at harvest was higher than bacterial coliforms. Additionally, the use of protected agriculture systems and good harvesting methods may have served to minimize contact of tomatoes with such environmental vectors.

Increased postharvest prevalence may have been due to increased contact between contaminated and non-contaminated products or surfaces, known as cross-contamination, or due to increased exposure in the environment. In the former scenario, in locations of increasing prevalence, the processes likely involve handling or facilitate contact between products or with surfaces. For example, the increase of TC prevalence between harvest and packing (OR=7.8) was somewhat counter-intuitive due to the chlorinated wash, but may be explained by the high contact with surfaces and other tomatoes in the packinghouse environment. The latter scenario suggests that wide spread concentrations in production or handling environments will be more widely transferred to products. For example, while none of the sampled tomatoes were visibly moldy or spoiled, increase of YM prevalence at the distribution center and retail supermarket (OR=9.0 and OR=51, respectively) may have been due to the use of circulated air to maintain the cold storage environment or the open environment in which they were displayed while on sale.

On the other hand, decreasing postharvest prevalence indicates complete removal of contaminated product or reduction of population levels to below the detection limit. For example, reduced prevalence of YM at packing may be due to the culling procedures or wash of product with chlorinated water. Reduced prevalence of TC during distribution may be due to the death or decrease of this population under the transportation and storage conditions (dry and low temperatures). Alternatively, since

the microbiological analysis in this study was made on surface microorganisms only, loss of prevalence may also have been due to non-detectable populations internalized through the stem scar *(18)*.

The increase in TC prevalence levels in the harvested lots between harvest and packing suggested that this step in the supply chain may be a significant risk area for spreading contamination. Conversely, the decrease in YM prevalence in lots between harvest and packing suggested that the packinghouse operations were better designed to control this fungal population. In both TC and YM prevalence, the supermarket was a significant risk area for spreading contamination. In addition to the retail practices and the older products, the period of greatest susceptibility to microbial decay onset is during ripening and senescence, which will occur at the end of the supply chain/supermarket *(1)*.

3.5.2 Concentration

Concentration, survival and growth of microorganisms on fresh produce are widely reported in isolated scenarios and various conditions from preharvest to retail. The change in concentration of indicator microorganisms on tomatoes moving through the supply chain was previously reported (Chapter 2) demonstrating this concept for the first time. In this study, the same concentration data were analyzed for significant spatial and temporal factors along the supply chain that explain changes within indicator populations in lots of tomatoes. The smaller model AIC values indicate the best fitting models between including or excluding days. Excluding the days factor gave location results similar to those previously reported for all three populations, showing an overall increase explained by the locations from harvest to supermarket. However, the results

when including the temporal factors, days and time of harvest, along with location altered the interpretation of how the supply chain influenced concentration. Each additional day the tomatoes were in the supply chain contributed between 0.4±0.1 and 0.5±0.1 log CFU/g, depending on the microbial population. Danyluk and Schaffner *(5)* reported literature data for *E. coli* O157:H7 on cut leafy greens, showing that during temperature abuse pathogens can increase by as much as 1 log CFU/g each day. The importance of maintaining the cold chain postharvest and after the packing house, as well as minimizing holding times may the primary risk preventive practices.

The seasonal effect, measured as time of harvest, did not predict YM or TC concentration and seems to be inconclusive along with other studies. Mukherjee et al. *(12)* looked at coliform counts on tomatoes based on production method—organic, semiorganic, or conventional—and reported ranges from 1.8-2.2 log MPN/g in homogenized tomato samples that did not differ over a two year period. Strawn et al. *(16)* reported that precipitation and temperature influenced *Listeria monocytogenes* and *Salmonella* prevalence in open fresh produce fields in New York State, but did not measure concentration. Alternatively, a study in South Africa reported that the level of coliforms on tomatoes at harvest was significantly higher due to climatic factors of the farm location (i.e. moderate temperature, high humidity, annual rainfall) *(17)*. In this study, the farm producing the tomatoes in Mexico utilized protected agriculture systems, such as greenhouses and shade houses, to reduce risks of contamination via such climatic or environmental vectors. There was, however, a reducing effect on APC towards the end of the harvest year, August through October. Nuevo León and southeastern Texas, the locations of the farm, packinghouse and distribution center, are

desert regions with relatively stable weather conditions. Most of the precipitation falls between August and February, albeit in low levels (average <13mm). The warmest months are June through September (29-32°C on average). So, the hot, dry conditions may have reduced the survival of microorganisms on tomato surfaces towards the end of the season. Additionally, the farm that was used for sampling in this study was preparing for a food safety audit in September, which may have altered practices during those months in a way that reduced microbial levels.

The effect of location along the supply chain, when days was included in the model, was decreasing across the three indicator microorganisms, with the exception of the packinghouse location for TC. The packinghouse practices and conditions neither increased nor reduced the level of TC ($p>0.05$), while for APC and YM there were significant reductions ($p<0.05$). In addition, the intercepts, which represent the level at harvest, were lower for TC and YM compared to APC, which may suggest the most likely sources of these populations on the tomatoes in this supply chain. Overall, APC and YM concentrations had similar changes over the locations (Figures 3.2 and 3.4). Furthermore, spoilage microorganisms (APC and YM) can exploit the tomato fruit defenses by using extracellular lytic enzymes that degrade structural polymers to release water and intracellular constituents of the fruit for use as nutrients for their growth; some are even capable of colonizing and producing lesions on healthy undamaged plant tissue at temperatures below 4°C *(1)*. A properly functioning washing system with chlorine between 50-200 ppm applied as a dip or spray to harvested fruit is capable of reducing the average APC by 10- to 100-fold *(1)*. While the samples in the packinghouse were not taken directly after the chlorine wash, the effect of the

packinghouse for APC, TC and YM was indicative of improperly functioning washing system or post-washing contamination. It is recommended to measure free residual chlorine concentration in wash water, instead of total chlorine. Lastly, while the effect of location decreased populations moving along the supply chain towards the supermarket, the increasing variation, as seen in the standard error, suggests that the supermarket could be an area for improved consistency in handling of tomatoes, food safety practices or further studies. Additionally, across the three indicator microorganisms, TC had the most variability at each location, making it a harder population to predict but also a risk indicator worth controlling.

3.6 CONCLUSIONS

From the results of this study, location practices were found to influence the cross-contamination and spread (prevalence) of microbial populations from products to surfaces, and vice versa, while temporal logistics greatly impacted the concentration of a microbial population on the product. More specifically, locations with increased prevalence of microbial indicators of food safety importance were the packinghouse and retail market and the difference in concentration between a six-day supply chain and a ten-day supply chain was often 2 log CFU/g. These findings suggest that best practices in the packinghouse and retail environment should focus on limited handling and frequent cleaning and sanitation of food-contact surfaces, and the design of shorter and more efficient supply chains may limit microbial growth on products.

While the microbial populations studied here are not foodborne pathogens and are loosely considered indicators of product safety and quality, the detectable levels enumerated from product surfaces throughout the supply chain allowed for observable

trends that have not been previously reported. Moreover, the sampling of one supply chain captured production from one farm and packinghouse, distribution through one shipping point, and sale at five US retail grocery stores. The observational study design captured the variable postharvest environments to which tomatoes were exposed. Previous controlled studies, reviewed herein, have reported a similar phenomenon important to outbreak behavior. Further research is needed to determine if these trends can be generalized to other supply chains and other fresh produce commodities. Here, the impact of current supply chain practices on the spread of microorganisms in tomato lots was quantified for use in further risk assessment models.

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CHAPTER 4

PRODUCE SUPPLY CHAIN WITH MICROBIAL TRAVELERS (PSCMT): MODELING TOOL AND GRAPHICAL USER INTERFACE

4.1 INTRODUCTION AND OBJECTIVES

This document describes the Produce Supply Chain with Microbial Travelers (PSCMT) modeling tool and its user-friendly graphical user interface (GUI). The PSCMT model was developed under the doctoral dissertation of Claire Zoellner, Cornell University Department of Food science, 2016. This model is a tool to understand the spread of microbial organisms through a fresh produce supply chain, to explore the impact of different contamination scenarios and intervention strategies, and to compile the vast amount of research in the area of microbial contamination of fresh produce. It is a multifaceted model that describes the dynamics of microbial contamination and features a graphical model of supply chain operations, a dynamic simulation program, and results analysis using pivot charts. The supply chain is taken to be a postharvest supply chain of fresh produce, meaning the handling operations encompass transportation of the harvested fruit or vegetable to a packaging location and subsequent activities to wash, cut, pack, store, distribute and sell the product.

Fresh produce supply chains are specifically designed to both preserve and monitor attributes of the product and its production environment due to its risk categorization and its high perishability. For example, as fresh produce is grown and harvested in an environment with many variables, minimally processed and packaged, transported long distances, and sold in the open fresh market, there are many opportunities for introduction or proliferation of microorganisms harmful to both the product (spoilage bacteria, yeasts and molds) and humans (pathogenic bacteria,

parasites and mycotoxin-producing molds). The postharvest handling activities do not include a definitive inactivation step, such as thermal processing, to reduce risks to near-zero. Previous research on prevalence of pathogens in the production environment, contamination risks during harvest, packing and cutting, along with temperature fluctuations during storage and distribution all suggest that there exists a research opportunity to trace the cumulative effect of these events on microbial populations in the route from farm to retail.

The goal of this generic model is to simulate the microbial dynamics of microorganisms in the supply chain of fresh and fresh-cut products as influenced by such handling operations. Understanding these dynamics can facilitate further development and testing of practices and interventions, with the ultimate goal of preventing outbreaks of foodborne illness. Because of the variety of pathogens that may contaminate fresh fruits and vegetables as well as the diversity in practices and supply chains employed, a proposed model must be flexible to capture differences in transfer, growth, survival, susceptibility to wash methods, etc.

For more detailed results of the model simulation and intervention testing results, the reader is referred to Chapter 5.

As the systematic needs for a microbial model in fresh produce supply chains have been discussed, in this documentation, we begin by describing the methods used to define the boundaries of the proposed supply chain model and its operations and annotate the product flow. Next, we introduce the dynamic microorganism flow and its attributes, which will be modeled in time steps through the given acyclic supply chain graph depending on specific operations and their parameters. Lastly, we explain how

the model has been implemented in a prototypical user interface for demonstration and simulation of example supply chain scenarios with user-input of model parameters. The model files are available upon request (cez23@cornell.edu).

4.2 MATERIALS AND METHODS

The model described below has been implemented in the JavaScript language with a prototypical user interface in hyper-text markup language (HTML), employing jQuery libraries such as EasyUI, JointJS, Pivot.js, fileserver.js, and others, under their non-commercial licenses. The initial software prototype was developed by Professor Peter Jackson, Cornell University Operations Research and Information Engineering, and the Systems Engineering Program.

4.2.1 Supply chain model

Let $i \in I$ index the food produce handling operations in the supply chain. Let $i = 0$ denote any source or sink operation outside of the supply chain, that is, the *outside world*, and let $I_0 = I \cup \{0\}$, the set of supply chain operations supplemented with the outside world.

Let $(i,j) \in I_0 \times I_0$ denote a directed product flow from operation i to operation j.

Let A_0 denote the set of all directed product flows, (i,j), in the supply chain, including flows to and from the outside world.

Let $A = A_0 \cap (I \times I)$ denote the product flows strictly internal to the supply chain.

The graph $G = (I, A)$ is assumed to be acyclic and directed.

For any operation i, let $S(i)$ denote the set of operations which receive directed flows from operation i. $S(i)$ is called the *successor set* of i. Let $P(i)$ denote the set of operations which send directed flows to operation i. $P(i)$ is called the *predecessor*

set of i.

Let $\alpha_{ij} \in [0,1]$ denote the yield of operation i giving rise to product flow to operation j. For conservation of mass, we must have

$$\sum_{j \in S(i)} \alpha_{ij} \leq 1,$$

that is, the sum of yields out of an operation cannot exceed 1.

Let λ_{ij} denote the rate of flow (with units such as grams per hour) of product from operation i to operation j and let λ_i denote the total rate of flow of product into operation i, $i \in I$:

$$\lambda_i = \sum_{j \in P(i)} \lambda_{ji}.$$

It follows that for any operation $i \in I$, the rates of flow out of the operation must satisfy

$$\lambda_{ij} = \alpha_{ij} \lambda_i \qquad (4.1)$$

for all $(i,j) \in A$. Let $\overline{\lambda}_{0i}$ denote the known rate of flow for product from the outside world to operation i.

Proposition. Given $\overline{\lambda}_{0i}$ for all $i \in I$, and α_{ij} for all $(i,j) \in A_0$ such that $i \in I$, there is a unique solution to the system of equations (4.1).

Proof: The graph G is acyclic, so the set of operations I can be partitioned into those that have no incoming arcs and those that do. Since G is acyclic, there is at least one such operation. Let I_1 denote the set of operations with no incoming arcs, except those from the outside world. Assuming only one arc connects the outside world with operation i, it follows that

$$\lambda_i = \overline{\lambda}_{0i}$$

for all $i \in I_1$. Then the relations *(4.1)* uniquely determine the flows on arcs connecting I_1 with the rest of the supply chain, $I \backslash I_1$. Now consider removing the operations I_1 from the graph. The remaining operations and arcs also form an acyclic directed graph. Let I_2 denote the subset of operations in the remaining operations which have no incoming arcs. It is easily seen that

$$\lambda_i = \overline{\lambda}_{0i} + \sum_{j \in I_1 \cap P(i)} \lambda_{ji}$$

for all $i \in I_2$. That is, the flows into operations in I_2 come either from the outside world or from operations in I_1. Now the relations *(4.1)* uniquely determine the flows on arcs connecting I_1 with the rest of the supply chain, $I \backslash (I_1 \cup I_2)$. The algorithm continues recursively (we next remove operations I_2 and identify operations I_3) until there are no remaining operations.

We imagine the flow of product to be continuous with no batch operations. That will prevent us from adequately modeling mixing and blending operations but it is a useful starting point.

4.2.2 Microbial flow models

In this section, we model the flow of microorganisms at a more detailed and dynamic level within each operation. Unlike product flows, which we view as stationary, our goal is to model the dynamic, non-stationary, transmission of microorganisms through the produce supply chain. We arbitrarily divide time into equal segments of length h, and number the time segments from the time of an initial event. We take the initial event to be a contamination at a specific point in the supply chain with a specified population of microorganisms. The goal of analysis is to predict the spread of these throughout the system. Let $t \in \mathcal{T} = \{1,2,\ldots,T\}$ index the time periods

from the initial event, $t = 1$, to the end of the predictive horizon, $t = T$. To describe conditions prior to the initial event, we use the index $t = 0$ and expand the time set to $\mathcal{T}_0 = \{0,1,\ldots,T\}$ to include these conditions.

4.2.3 Microbial flow in a single operation

First, we focus on a single operation, $i \in I$, and suppress the subscript i that would distinguish the variables of this section from those of other operations. For example, we take λ to be the flow rate, in grams, of the product through the entire operation. We distinguish between $N_{P,t}^{I}$, which is the number of microbes on product input to the operation in time segment t and $N_{P,t}^{O}$, which is the number of microbes on product output from the operation in time segment t.

4.2.3.1 Contamination and Removal

Given the nature of its production and environment and subsequent supply chain, fresh produce can become contaminated with microorganisms at a variety of points via a variety of mechanisms. The contamination of fresh produce can and has occurred during production, harvesting distribution and final preparation via mechanisms such as water, insects, rodents, and human handling, among others. For the most part, the human pathogens associated with fresh produce originate from the intestinal tract and fecal materials of animals and humans, or from soils and water *(15)*. Bacteria have been shown to preferentially attach to stomata or cut surfaces and infiltrate via stem scars and blossom or stem ends *(16)*. While different compounds may be used to sanitize fresh produce, once product is contaminated with bacterial or viral pathogens, these methods cannot guarantee safety and merely serve to reduce the number of pathogens. For these reasons, modeling the effect of management and

handling practices on these populations is of interest for minimizing consequences of such events.

In this model, **Contamination** is the introduction of microorganisms into the system from an external source. Regardless of the source, it can be defined as

$$N_{P,t}^{O} = N_{P,t}^{I} + n1_{\{t \in E\}}$$

where *n* is the *load*, which we define as the number of microorganisms arriving on the product in each time period of the contamination episode, *E,* and 1_A is an indicator of the event *A* ($1_A = 1$ is *A* is true and $= 0$ if *A* is false). A contamination episode is defined by a *start period* and a *duration*.

As microorganisms, both quality- and safety-associated, cannot be seen preemptively with the naked eye, there are other methods prior to packing of visually inspecting and removing products that are damaged and may detrimentally affect the lot. This practice is known as "culling" and includes removing split, damaged or rotting products that are thought to present nutritive niches in which microorganisms may survive, grow and leave by-products. Furthermore, food-contact surfaces in packinghouses are generally maintained under clean and sanitary conditions via routine application of cleaning agents and chlorinated compounds, thus removing microorganisms that may be present. Examples of sanitizing compounds include sodium hypochlorite, calcium hypochlorite, acidified calcium hypochlorite, sodium bromide, chlorine dioxide, chlorine gas, hydrogen peroxide, ozone, and organic acids, all of which are oxidizing compounds that are biocidal. Reductions observed over a wide range of conditions on products and surfaces are generally between 0 and 1,000-fold.

In this model, the operation **Removal** is defined as the physical elimination of products and/or microorganisms from the system flows. It is assumed that heavily contaminated units are not removed with higher probability. Due to the nature of removal studies and reported reductions in logarithmic scale, removal is modeled as

$$N_{P,t}^{O} = N_{P,t}^{I}/(10^{n} * 1_{\{t \in R\}})$$

where *n* is the *load*, i.e. the log reduction or number of microorganisms, removed from the product in each time period of the removal episode, *R*, and 1_A is an indicator of the event *A* ($1_A = 1$ is *A* is true and $= 0$ if *A* is false). The removal episode is given by a *dwell time* of the product in the operation.

4.2.3.2 Survival and Growth

It is known that pathogenic bacteria will survive but will not divide and multiply on the uninjured outer surface of fresh fruits and vegetables, due to protective barriers native to plant physiology (i.e., cell walls and wax layers), especially if the humidity is high *(15)*. Foodborne pathogens do not have the enzymes required for breaking down these protective barriers and releasing nutrients necessary for growth. Therefore, in some cases pathogen levels will decline on this outer surface depending on the organism, product and conditions. As this process varies and microorganisms adapt to conditions (form spores or biofilms), it is possible that microorganisms persist in food processing environments if proper sanitation is not followed. **Survival** is defined as the stagnant or slow decline of the microorganism population given the environmental conditions, for example desiccation or UV inactivation from sunlight exposure.

Survival is enhanced in the event that the plant barriers are broken, either by physical puncturing or bruising or by bacterial or fungal degradation, thus releasing

sources of energy. Under the right temperatures, surviving microorganisms on the surface of products or equipment will have conditions suitable for growth and multiplication. Therefore, **Growth** is defined as the exponential increase in the microorganism population per day due to cell division and multiplication.

The basic model for survival or growth on the product is:

$$N^{O}_{P,t+d} = (N^{I}_{P,t})e^{\gamma d}$$

where γ is the daily *microbial growth rate* (resp. *survival rate*) if $\gamma > 0$ (resp. $\gamma \leq 0$) and d is the *dwell time* in this operation. This model could also be expanded to include a *lag time*, if appropriate.

4.2.3.3 Transfer

Following the initial contamination event, the spread of microorganisms throughout the system is influenced by surfaces and their characteristics *(6-9, 22)*. **Transfer** is defined as the cross-contamination of microorganisms from one surface to another within the system, either a unit of produce or a food-contact surface.

The transfer operation is conceived of as a linear conveyor divided into discrete sequential segments to articulate the interaction between the contaminated product and the food-contact surfaces of the supply chain. Let $k \in \mathcal{K} = \{1,2,\ldots,K\}$ index the discrete segments, where K is the total number of segments considered. The dwell time of product in each segment is exactly h, the time step of our model. Consequently, the total dwell time of product in the operation is given by hK. We imagine that all product in segment $k \in \mathcal{K}$ at time t moves into segment $k+1$ (or in the case of $k = K$, moves out of the operation) during time segment t and is replaced at time $t+1$ by the product which was in segment $k-1$ (or, in the case of $k = 1$, by input

from the previous operation(s)).

Each segment has a set of time-denominated attributes B_{kt} which we track cumulatively over the dwell time in the supply chain. These attributes measure such things as, N_P, the number of microorganisms on the product in segment k at time t and, N_S, the number of microorganisms on the equipment surface in segment k at time t. We could also denote these quantities with the appropriate subscripts as $N_{P,k,t}$ and $N_{S,k,t}$, respectively. These attributes are updated according to the operation parameters, defined below

$$N_{P,k+1,t+1} = (1 - \alpha_k)N_{P,k,t} + \beta_k N_{S,k,t}$$

$$N_{S,k,t+1} = (1 - \beta_k)N_{S,k,t} + \alpha_k N_{Pk,t}$$

where α_k is the *deposition rate* of microorganisms from product to surface and β_k is the *contamination rate* from surface to product with regard to surface in segment k. The input level of microbes in the product along the arc coming from the previous node is immediately entered into the first segment of the transfer function. Similarly, the overall output level of microbes in the product that will pass along the arc leading to the subsequent node is derived from the level in the final segment of the transfer function. This is defined as follows

$$N_{P,0,t} = N_{P,t}^{I}$$

$$N_{P,t}^{O} = N_{P,K,t}$$

Removal from food-contact surfaces by means of regular cleaning and sanitation can also be modeled and will impact the dynamics of the population leaving a packinghouse, for example. It is incorporated into the model as

$$N_{S,k,t} = 0, when\ t\ \%\ j == 0$$

for all segments $k = 1,2, \ldots, K$, where j is the *rejuvenation or cleaning cycle* and $t \,\%\, j$ is the modulus of t and j ($t \,\%\, j == 0$ only when t is an integer multiple of j). By this mechanism, the surface population is set to zero during repeated intervals of this cycle.

Furthermore, following a transfer event, microorganisms on food-contact surfaces may have the opportunity to survive or grow prior to or in conjunction with subsequent transfer events. This can easily be added into the transfer model

$$N_{S,k,t+1} = \left((1-\beta_k)N_{S,k,t} + \alpha_k N_{Pk,t}\right) * e^{\Upsilon_k}$$

where again, γ_k is the *microbial growth rate* (resp. *survival rate*) if $\gamma_k > 0$ (resp. $\gamma_k \leq 0$) for microbes on surface segment k.

4.2.4 Microbial Concentration

The attributes being modeled in the continuous flow are defined simply as the number of microbial cells either in product, N_P, or on equipment surfaces, N_S. So, in order to report this more conventionally as a microbial concentration on product ($C_{P,t}$, cells per gram), N can be linked to the previously defined product flow rate, λ_i (grams per period). Because the flow rate λ_i is constant,

$$C_{P,t} = \frac{N_{P,t}^{O,i}}{\lambda_i}$$

4.2.5 Microbial Flow at Junction Points

Suppose there are two operations in series, $i, j \in I$, with $(i,j) \in A$, and no other arcs emanating from operation i or entering operation j. There will be a transfer of microorganisms from operation i to operation j at each time step of the model. Let $N_{(i,j)t}$ denote the number of microorganisms to be transferred along arc (i,j) during time step t. To align this flow with the flow of microorganisms within each operation,

we need to distinguish the microbial counts by operation index. For this purpose we will use a superscript i or j. Hence, $N_{P,t}^{I,j}$ is the number of microorganisms entering operation j in time step t and $N_{P,K,t}^{O,i}$ is the number of microorganisms leaving operation i in time step t. From this, we define the transfer as instantaneous:

$$N_{P,t}^{I,j} = N_{(i,j)t} = N_{P,t}^{O,i}.$$

4.2.5.1 Mixing and Fractionation

Certain operations act to merge or split product flows from or into separate product streams. The product flows are captured by the graph $G = (I, A)$ described in section 4 above. For any operation,$i \in I$, if there are incoming arcs from other operations, we assume that the mixing or blending occurs in the first segment of operation i. **Mixing** is defined as the combination of two or more product flows, and the associated microbial flow, into a single supply chain operation.

Likewise, if there are any outgoing arcs to other operations, we assume the splitting occurs in the last segment of operation i. **Fractionation** is defined as a split of the product flow, and its associated microbial flow, into multiple flows or when a large unit becomes several smaller units *(19)*.

With these processes, the unit size or product flow is modified and the associated microbial flows are reallocated, updating the number of cells per unit *(19)*. We assume uniform, homogeneous prevalence of microorganisms within the product. Consequently, if there is a split in product flow, we assume a proportional split in bacterial flow:

$$N_{(i,j)t} = \frac{\lambda_{ij}}{\sum_{k \in S(i)} \lambda_{ik}} N_{P,t}^{O,i}$$

That is, we divide the microbial population across arcs leaving operation i in proportion to the product flow rates on these arcs. The number of microorganisms entering an operation j is simply the sum of the transfers on all incoming arcs:

$$N_{P,t}^{I,j} = \sum_{k \in P(j)} N_{(k,j)t}.$$

4.3 RESULTS

Foodborne illness resulting from consumption of fresh produce is dependent upon the factors included in the operations above. In short, the product must be contaminated, the pathogen must survive, and the level of pathogen consumed must be sufficient to cause illness in the host, a level which is very low for many pathogens. Therefore, with this generic model, experimentation with several postharvest supply chain scenarios and suggested interventions is interesting for simulation of the microbial levels in the final product presented to the consumer. The scenario must always be initiated by contamination, but the model framework is flexible to allow contamination at any point in the supply chain and then to also reoccur at subsequent locations. For example, the schematic representation in Figure 4.1 below describes a fresh tomato supply chain of interest and for demonstration of the flexibility and complexity required to capture such microbial dynamics. The generic supply chain involves the field, packinghouse, distribution center, and retail supermarket. And, the basic microbial processes that could occur along the way are those encompassed within the model nodes: contamination, removal, transfer, growth/survival, fractionation, and mixing. Fresh-market tomatoes have a notably non-traditional marketing channel due to their ripening process that can be adjusted through modification of time variables in each location. The "example scenarios" box describes how this generic supply chain can be

modified to encompass the wide variety of practices used (field packing, dump tanks, spray washing, co-packing) and supply chains (direct to market, distribution centers, retail) involved with fresh tomatoes.

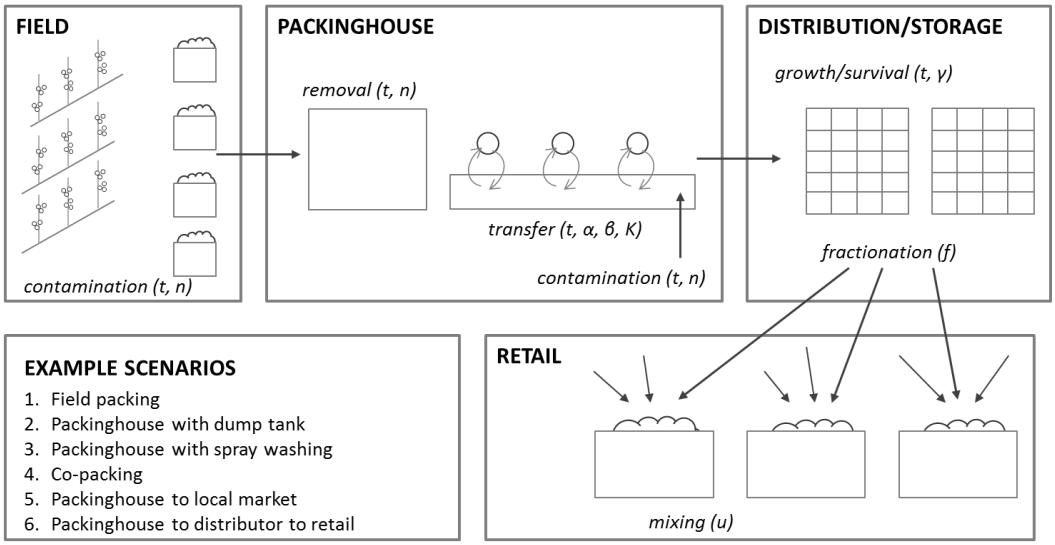

Figure 4.1 Schematic representation of potential postharvest processes simulated by the model

4.3.1 Structure of the GUI

The user interface is a tool entitled **Produce Supply Chain with Microbial Travelers (PSCMT)** and features a graphical model of supply chain operations and microbial spread, a dynamic simulation program, and results analysis using pivot charts. The on-line tool consists of a tabbed layout with five screens: *Welcome, Model, Run Controls*, *Results* and *References*. Additionally, there are two drop-down menus (File and Tools) for saving or opening files, running validation checks on a designed model prior to running, and exporting results. The *Welcome* screen explains the basis and features of the modeling tool and how to set up a simulation run, referring users to this document for more detailed guidance.

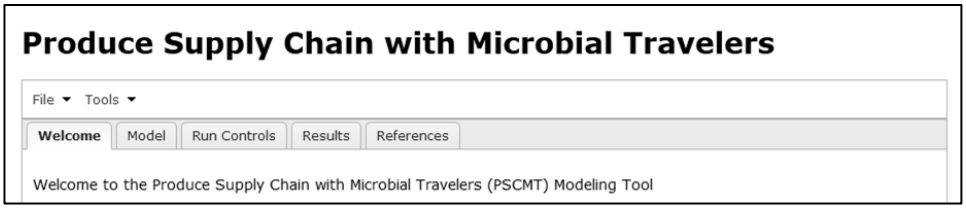

Figure 4.2 The tabs included in the graphical user interface of PSCMT

4.3.2 Using the graphical editor: an example scenario

The *Model* screen provides the canvas for building and modifying a supply chain using a *graphical editor* to later simulate. Use the File→Open menu item to load a pre-existing, tested model or the File→New menu item to clear an existing model and build your own. The model is structured as a graph with nodes and links. Therefore, the *graphical editor* allows for addition, editing and deletion of the model nodes and links, which can be seen in the simple diagram below. To create nodes, right-click on a blank area and select the type of microbial operation to add. The nodes are connected by arcs that are labeled with the *flow fraction* that will proceed along the arc to the next node. Drag and drop the nodes onto one another to establish the sequence of links that form the desired supply chain. Right-click on the arc to edit the flow fraction. For example, in Figure 4.3a below, all flow fractions are equal to one, so the flow is conserved along this system. The flexibility of this model and *graphical editor* allows the user to specifically design the appropriate supply chain, which may in fact be a web of farms, packinghouses, distribution centers and retailers. In Figure 4.3b, there are two nodes ("farms") that flow into the packinghouse and two nodes ("distribution") that receive packed product to demonstrate different flow fraction scenarios that make a supply chain a network.

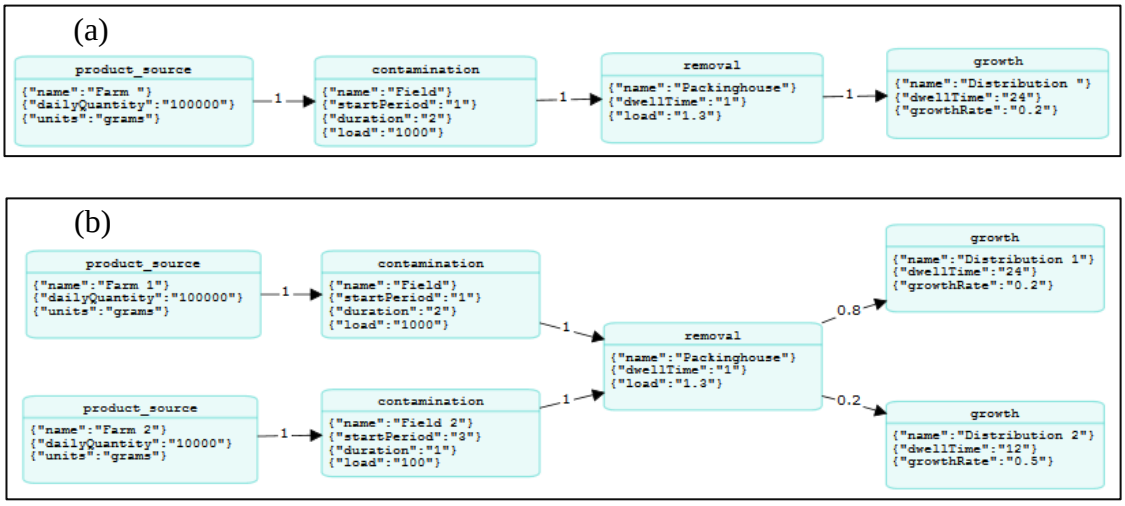

Figure 4.3 (a) Simple linear diagram of nodes and arcs, and (b) Acyclic diagram of nodes and arcs made with the graphical editor

4.3.3 Setting and changing parameters

Once the model's graphical structure is set, editing of the nodes to set or change default parameters can be done by right-click. Each node has a process name (i.e., contamination, etc.), to describe the microbial operation being modeled, and a descriptive name (i.e., harvest, retail, etc.), to describe the location in the supply chain. Additionally, within each node are the parameters required to model the associated microbial behavior (described in detail in Section 4.2) that must be determined by the user, although there are default values given. The *Resources* screen provides a comprehensive database of parameter estimates collected from literature and modeling sources for fresh produce and the pertinent microorganisms and supply chain scenarios. The current version of the model only allows for parameter values to be point values or plain text. Use the Tools→Validate menu item to run a sequence of validation checks on the model. This may uncover errors in the model format or parameter input that will prevent the model from running correctly and will give alerts accordingly.

4.3.4 Running the model

The *Run Controls* screen contains an area for a model description to be saved with the model file, as well as controls for measuring and simulating time. The user should set the controls for the simulation, such as how many periods are in a day and how many days to simulate the supply chain. When the model is ready for simulation, and after the validation step has ensure that all parameters, nodes and arcs have been specified correctly and completely, run the simulation and execute the model from this screen.

4.3.5 Visualization of results

Upon execution of the model, the screen switches to the *Results* screen where a Pivot chart displays a plot of the microbial levels over time for each node of the supply chain. Because it is a Pivot chart, the axes and chart type can be modified to display the data differently. The top bar lists all of the attributes found in the results data. The second to top bar lists the current attribute selected for the x axis of the chart. The left bar lists the current attribute selected for the different time series to plot. A drop-down box allows you select which attribute to plot and aggregate over, either 'value', 'cumvalue', 'concentration'. When the axis is per time period, 'logvalue' may also be chosen, however not when using the day view. A drop-down box allows you to select the plotting style. By selecting File→Save As from the toolbar, the model can be saved as a JavaScript Object Notation (.json) file for future study or analysis. Selecting File→Export Results As will save the simulation results as a Comma Separated Values (.csv) file to be used in Excel for any other analyses.

The corresponding results of the model in Figure 4.3b are shown in Figures 4.4

and 4.5 as a plot of value (microorganisms) versus day in the supply chain as line and bar charts, respectively. The lines and bars represent the named nodes of the system. While the contamination is introduced at harvest (yellow) and is reduced at washing (purple) in the first day, the co-mingling of product, split of the flow across two distribution centers, and growth functions during distribution (red and blue) spread out the microbial level for non-contaminated products over the next 4 days. The line and bar charts allow for visualization of where the initial contamination is along the supply chain over the simulation time period.

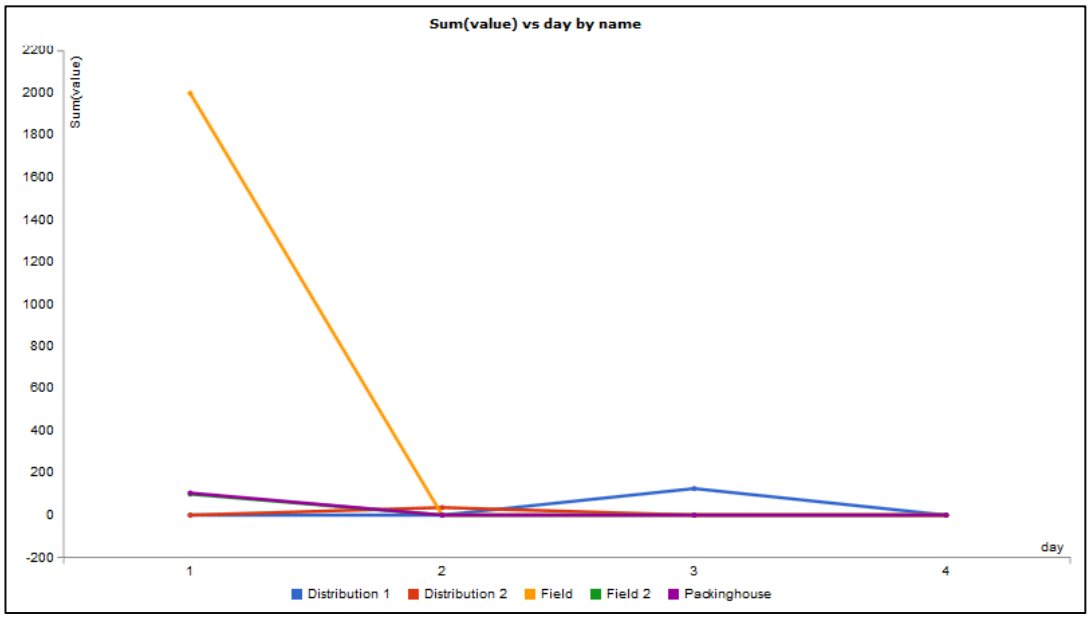

Figure 4.4 Line chart of results of the example supply chain scenario by day

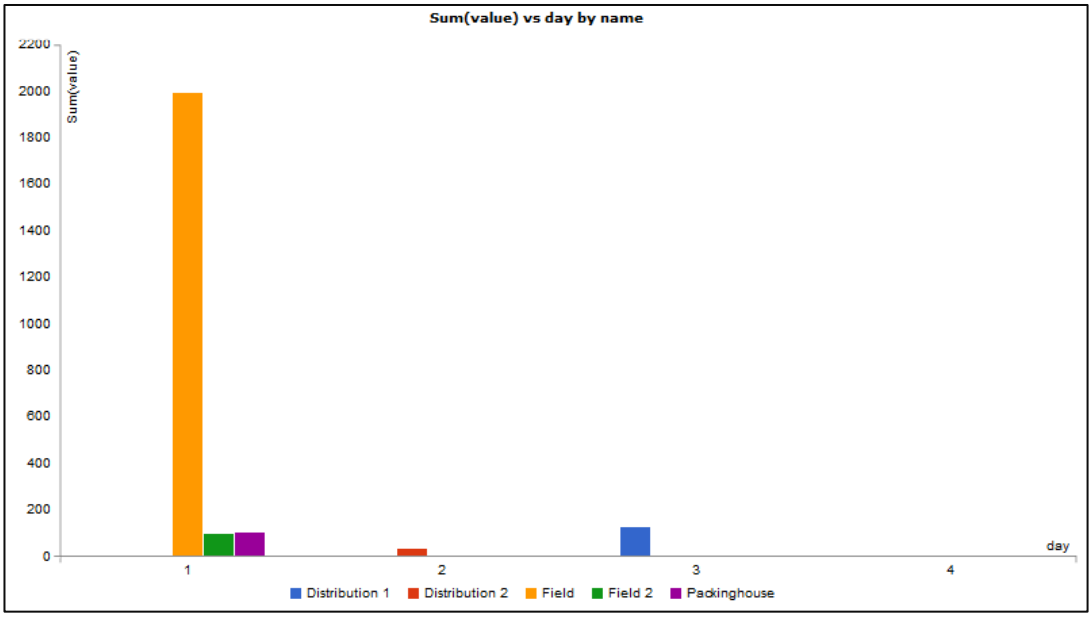

Figure 4.5 Bar chart of Results of the example supply chain scenario by day

4.3.6 Limitations

From the above example use of the model, the advantages and disadvantages of the detailed mechanistic model are displayed. This modeling tool is highly flexible for simulating a wide range of interventions and processes in the postharvest supply chain of fresh produce. However, this comes at the expense of the amount of data needed to describe specific supply chains and microbial hazards and parameterize the model. This data need relies on existing published studies and tools, reports from outbreak investigations or personal environmental testing results.

For example, starting levels of contamination may be inferred from previous outbreak investigation data or from environmental swabbing of the production environment. Additionally, the load of microorganisms introduced in the contamination episode can be consistent with levels traditionally used in research experiments. Growth and survival rates can be obtained from controlled studies *(1, 2, 4, 5, 23, 25, 27)*

or database programs such as PMP and ComBase (Section 3), but are generally limited to broth media, laboratory cultures and products requiring secondary growth models. There are a select number of papers describing growth of pathogens on the surface of damaged produce but differing inoculation methods and incubation conditions make it difficult to assess the precision of the model parameters. Transfer studies are confined to certain pathogen/surface material/produce combinations *(1, 7-9, 12, 22, 24)*and are limited by inconsistencies of methods and reporting formats. However, as the focus of produce safety zooms in on packinghouse design, further study of these transfer parameters will be forthcoming. Lastly, removal from washing via dump tanks or spray and brush systems have been studied on several produce commodities and the efficacy of chlorine compounds is well-reported *(3, 11, 21, 26-28)*. Data for mixing, fractionation, and dwell times will be best taken from the user's personal experience in their supply chain.

For assistance with parameter-fitting, default parameter values are given upon creation of a node and **PSCMT** includes a *Resources* screen with a comprehensive collection of parameters for fresh produce items and their handling or processing steps, but still may not encompass every scenario desired. The amount of required parameters to run the model may therefore be limited by availability and knowledge of appropriate resources for parameter estimation. Furthermore, in the event that resources do not exist, the model will be limited by assumptions or arbitrary assignment of parameters.

Finally, several aspects of QMRA or other similar models remain out of the scope of the **PSCMT** modeling tool. For example, the model is purely deterministic, so there is no stochasticity for evaluating variation or uncertainty of model parameters.

Similarly, it is not a complete QMRA, but merely an exposure assessment as a preliminary step in the QMRA process. The output of the model could be an input into existing dose-response modeling tools, if the risk of illness is desired. Lastly, as the model simulation of concentration is already complex and does not incorporate stochasticity, prevalence is not an included attribute of the product flow. It could be a future improvement to the model as the current version gains acceptance.

4.4 CONCLUSIONS

The irregular distribution, in time and location, of contaminated fresh produce has pushed the philosophy of its characterization towards two parameters, concentration and prevalence. The ability to quantify these parameters is a current research endeavor for risk assessment of the supply chains that make up fresh produce production systems. The complex nature of microbial populations, variability and detection, in the environment is a challenge to accurate mathematical definitions. Therefore, here we present a dynamic simulation program, **PSCMT**, based on detailed deterministic equations that describe microbial behavior to estimate how the microbial flow changes due to postharvest handling and operations.

The deterministic equations encompass microbial, physical, chemical and operational behaviors related to the supply chain. For example, growth and survival are intrinsic behaviors to the microbial population; transfer and removal depend on physical features of the packinghouse; removal may also be defined by chemical activity of sanitizers against cells; and fractionation and mixing allow for logistical flows of the supply chain. The user-oriented tool was developed to allow for customizable supply chain scenarios and/or network of supply chains, which may be more representative of

today's food system. In addition, its transparency provides the opportunity to understand how the parameters chosen affect overall population behavior. The output given as either number of microorganisms or concentration in the Pivot Chart can further help in determining optimal intervention strategies along the supply chain.

In conclusion, the goal of this generic model was to describe the microbial dynamics of microorganisms in the supply chain of fresh and fresh-cut products as influenced by such postharvest handling operations. Construction of this mechanistic model provides a method for conceptual understanding of such transmission dynamics as a result of a given system design. With this understanding, further development of practices, research interventions and mathematical simulations are made possible, with the ultimate goal of preventing outbreaks of foodborne illness.

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CHAPTER 5

A FARM TO RETAIL SYSTEM DYNAMICS MODEL FOR CONTROL OF *SALMONELLA* ON FRESH TOMATOES

ABSTRACT

Risk assessment modeling tools are useful in studying outbreaks of foodborne illness, despite the rare occurrence and low prevalence of pathogens. The proposed Produce Supply Chain with Microbial Travelers (PSCMT) is a step forward in risk assessment of fresh produce supply chains. Here the objective was to demonstrate the capabilities of the PSCMT tool by recreating microbial levels in an observed supply chain. Parameter values for transfer, growth/survival, removal and contamination events were taken from existing published studies. The working model of the PSCMT supply chain was optimized to obtain estimated log CFU values within one standard deviation of the observed aerobic plate count data. Subsequent simulations were designed and run to test different packing methods, contamination events, temperature abuse, and packinghouse conditions. When chlorinated water was used, there was no difference in levels from the dump tank and spray wash packing methods. Point-source and persisting contamination events present different foodborne illness outbreak patterns. Here, point-source contamination showed more transfer between surfaces in the packinghouse and more microbial flow, while persistent contamination presented less contamination in each period, allowing for more efficient removal during washing. Growth during postharvest holding of wounded tomatoes with 2.0 log contamination resulted in levels entering and leaving packing as 2.3 and 2.0 log, respectively. Temperature abuse during distribution of packed tomatoes with 1.9 log contamination increased to 4.1 log and 4.4 log at distribution and retail, respectively. Lastly,

simulations of food contact surface materials showed that larger alpha and gamma values removed more cells from the flow and resulted in less cumulative output as fewer microorganisms were surviving on the surface in each period. Larger beta values resulted in fewer periods of contamination output and a higher cumulative flow out of the transfer, across all materials, cleaning frequencies and contamination events. While this modeling tool provides data that may be incorporated in an exposure assessment, emphasis should be placed on the relative changes in contamination. Insight into the microbial dynamics may provide produce growers, handlers and retailers some direction in focusing risk mitigation strategies.

5.1 INTRODUCTION

The CDC estimates that of the 48 million Americans that become ill each year due to contaminated food, 46% will have consumed fresh produce that has been contaminated with pathogens such as Norovirus, *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes*, among others *(11, 20, 22)*. Furthermore, 38% of hospitalizations and 23% of deaths from foodborne illness are attributed to fresh produce. The rare occurrence of outbreaks (in consideration of overall consumption) and low prevalence of pathogens make these outbreaks difficult to predict and study.

Risk assessment models and software tools are useful in studying such events because of the ability to test many variables and hypothetical situations utilizing existing research, with a relatively inexpensive design *(26)*. Detailed and mechanistic models allow for both conceptual understanding of contamination dynamics during the entire production process and estimations of the impact of alterations to the process design. These models can inform policy, focus and/or allocate resources, and guide future research and data collection. Similar models exist in other food commodities and supply chains such as fresh pork *(24)*, leafy greens *(13, 14)*, cheese *(3)*, poultry *(19)*, and berries *(18)*. Several examples of these tools were also listed in Chapter 1 and the limitations when applied to fresh produce were discussed. The proposed PSCMT in Chapter 4 addresses some of those limitations and is a step forward in risk assessment of fresh produce supply chains.

Although it has been recognized that these quantitative microbial risk assessments overestimate the number of cases of foodborne illness when calculated, it is suggested to place more emphasis on the relative risks when making management

decisions *(24)*. Here the objective was to demonstrate the capabilities of the PSCMT tool by recreating the supply chain and microbial levels from Chapter 2 using both observed and literature values. Replicating the supply chain environment is one method of validation and also allows for testing effects of contamination scenarios or intervention strategies in certain locations along the supply chain. Utilizing behavior that is known about *Salmonella* and indicator organisms on tomatoes under laboratory conditions and conditions of the postharvest supply chain may suggest potential risk areas. As the overall output of the model is concentration at retail, the results could be used in combination with other risk models with dose-response capabilities.

5.2 MATERIALS AND METHODS

5.2.1 The simulation model

The Produce Supply Chain with Microbial Travelers (PSCMT) modeling tool implemented in the JavaScript language with a prototypical user interface in hyper-text markup language (HTML) described in detail in Chapter 4 was applied to the supply chain of fresh tomatoes studied in Chapters 2 and 3. The tool was used to estimate the contamination level at the postharvest handling, distribution, and retail locations. It allows for introduction of contamination in the field, but also at subsequent locations in the supply chain. The flow of product (fresh tomatoes) was assumed to be continuous with no batch operations. Steps of the supply chain were described by the microbial nodes of the tool: contamination, removal, transfer, and growth/survival. Each operation alters the associated microbial flow (*Salmonella*) according to the relevant equations previously described and parameters estimated from literature values. First, a working model of the aerobic plate count levels observed in the supply chain was

created to select the appropriate parameters to explain the conditions and microbial behavior. A literature review was then conducted to summarize the existing data for relevant model parameters for behavior of *Salmonella* and indicators on tomatoes. Figure 5.1 provides a copy of the observed values from Chapter 2.

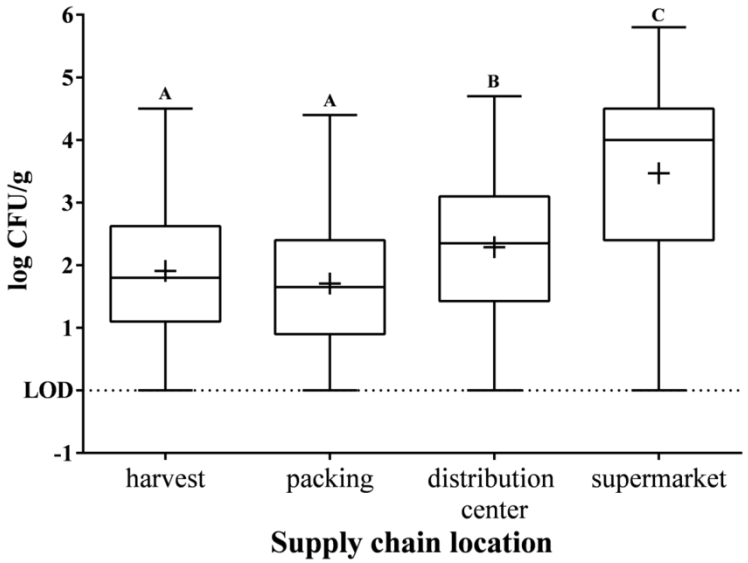

Figure 5.1 Copy of observational study results for aerobic plate counts on tomatoes moving through the supply chain from field to supermarket.

5.2.2 Data Sources

Research on parameter values for transfer, growth/survival, removal and contamination events are listed in Tables 5.1-5.5. The inverse of reported log % transfer coefficients were taken to give alpha and beta parameter estimates relevant to the microbial flow. Similarly, growth and survival rates (gamma) were converted for use in the exponential growth model used in PSCMT. For parameters lacking experimental data, reasonable estimates were made within the expected range of the parameter and noted.

Table 5.1 Transfer of *Salmonella* from tomatoes to different surfaces (α)

Tomato Attributes	To surface of:	Conditions	Log Transfer (mean±std)	α	Reference
mature, green, round	reusable glove	wet	0.18±0.0	0.015	*(9)*
		dry	0.38±0.2	0.02	
	single-use glove	wet	0.37±0.2	0.02	
		dry	0.39±0.2	0.02	
	clean cotton cloth	20-s touch, wet inoculum	0.37±0.07	0.02	*(25)*
		20-s touch, dry inoculum	0.04±0.06	0.01	
	dirty cotton cloth	20-s touch, wet inoculum	0.39±0.13	0.02	
		20-s touch, dry inoculum	0.05±0.06	0.01	
red, round	dump tank	2 min wash, 3 tomatoes (~6 log cfu/g)	0.35±0.1*	0.02	*(27)*
	water recirculation tank	2 min wash, 3 tomatoes (~6 log cfu/g)	0.28±0.1*	0.02	
	plastic roller conveyor	immediately after washing inoculated tomatoes (~6 log cfu/g) in dump tank	0.34±0.1*	0.02	
	plastic roller conveyor	53 polyethylene rollers, 10 inoculated tomatoes (3 log cfu/g) passed over rollers	0.53±0.1*	0.03	*(28)*
	foam roller conveyor	19 latex rollers, 10 inoculated tomatoes (3.3 log cfu/g) passed over rollers	0.69±0.03*	0.05	
	brush roller conveyor	26 brush rollers, 10 inoculated tomatoes (3.1 log cfu/g) passed over rollers	0	0	

*TC estimated from figure data

Table 5.2 Transfer of *Salmonella* from surfaces to tomatoes (β)

Tomato Attributes	From surface of:	Conditions	Log Transfer (mean±std)	β	Reference
mature, green, round	reusable glove	clean, wet inoculum, contact 5s	0.25±0.1	0.02	*(9)*
		clean, dry inoculum, contact 5s	0.48±0.5	0.03	
		dirty, wet inoculum, contact 5s	0.41±0.3	0.03	
		dirty, dry inoculum, contact 5s	none detectable (enrichment)	0	
	single-use glove	wet inoculum, contact 5s	0.32±0.1	0.02	
		dry inoculum, contact 5s	0.29±0.2	0.02	
red, round	plastic roller conveyor	53 polyethylene rollers, 10 inoculated tomatoes (3 log cfu/g) passed over rollers followed by 25 uninoculated tomatoes	0.013±0.008	0.01	*(28)*
	foam roller conveyor	19 latex rollers, 10 inoculated tomatoes (3.3 log cfu/g) passed over rollers followed by 25 uninoculated tomatoes	0.18±0.09	0.01	
	brush roller conveyor	26 brush rollers, 10 inoculated tomatoes (3.1 log cfu/g) passed over rollers followed by 25 uninoculated tomatoes	<0.001	<0.01	
Roma, skin	polyethylene roller brushes	2 brush rollers, spray inoculated (6.9 log CFU/cm^3), 6 non-inoculated tomatoes passed over with just brushing, 60s	0.83	0.07	*(21)*
		2 brush rollers, spray inoculated (6.9 log CFU/cm^3), 6 non-inoculated tomatoes passed over with water spray, 60s	0.48	0.03	
		2 brush rollers, spray inoculated (6.9 log CFU/cm^3), 6 non-inoculated tomatoes passed over with 5 ppm chlorine dioxide spray, 60s	0.10	0.01	

Table 5.3 Growth or survival of microorganisms on whole and cut tomatoes (γ)

***Salmonella* serotype**	**Tomato Attributes**	**Inoculation Method**	**Temp (°C)**	**Specific growth rate (d^{-1})**	**Ref.**
S. Montevideo	cut slices	Spot inoculation with 25ul in water on each slice (3.4 log CFU/slice)	25	9.4	*(29)*
	fully ripe, wounded	Spot inoculation on each wound (8 spots: 2mm x 0.6cm), 3 log CFU/wound	25	4.6	
	mature green, wounded	Spot inoculation with 25ul on each wound (8 spots: 1mm x 0.6cm), 3 log CFU/wound	25	4.1	
	stem scar	spot inoculation with 25ul in water on stem scar, 8 log	20	-0.5	
			25	-0.7	
		spot inoculation with 25ul in TSB on stem scar, 7.2 log	20	-0.9	
			25	-0.3	
	skin	spot inoculation with cells in water, saturated filter disks for 2h, 5.8 log	20	-2.2	
			25	-3.7	
		spot inoculation with cells in TSB, saturated filter disks for 2h, 5.5 log	20	-1.0	
			25	-0.5	
S. Typhimurium, *S.* Infantis, and *S.* Enteriditis	cut, small pieces	0.1ml cell suspension inoculated into 20g samples sealed in PE plastic bags	7	0	*(2)*
			22	12-13	
			30	14-17	
S. Montevideo	mature, green, skin	dip inoculation, stored individually in open plastic bags (relative humidity 45-60%)	10	0.1	*(33)*
			20	1.5	
			30	1.7	
	ripe, chopped	1ml cell suspension inoculated into 50 g sample	5	-0.05	
			20	4.0	
			30	6.5	
cocktail of: *S.* Agona, *S.* Baildon, *S.* Gaminara, *S.* Michigan, and *S.* Montevideo	round, stem scar	spot inoculation with 0.02 ml cocktail	12	0.4	*(8)*
			21	1.1	
	Roma, stem scar	spot inoculation with 0.02 ml cocktail	12	0.3	
			21	0.8	
	grape, stem scar	spot inoculation with 0.02 ml cocktail	12	0.5	
			21	0.5	

Table 5.3 continued Growth or survival of microorganisms on whole and cut tomatoes (γ)

S. Montevideo	green/unripe, skin	dip inoculation of tomatoes (23°C) in suspension (5°C, 10^6 CFU/ml) for 10 min, followed by 3 vacuum-release cycles to facilitate internalization	25, 75%RH	0.9	*(23)*
			25, 95%RH	1.0	
			15, 75%RH	0.8	
			15, 95%RH	0.7	
	red/ripe, skin	dip inoculation of tomatoes (23°C) in suspension (5°C, 10^6 CFU/ml) for 10 min, followed by 3 vacuum-release cycles to facilitate internalization	25, 75%RH	0.9	
	red/ripe, skin		15, 75%RH	0.8	
S. Montevideo	red/ripe, skin	spot inoculation (5 log CFU/fruit) near blossom end	30, 60%RH	0.2	*(16)*
			30, 75%RH	0.2	
			30, 85%RH	0.3	
			30, 97%RH	0.5	
			22, 60%RH	0	
			22, 75%RH	0.2	
			22, 85%RH	0.2	
			22, 97%RH	0.3	
aerobic mesophiles, total coliforms, and yeasts/molds	roma, skin	observed from supply chain	--	0.3	*(34)*
		observed over 10d storage	10, 90%RH	0	
aerobic mesophiles	cherry, skin	observed on tomatoes purchased a retail and stored for several days, no chlorine treatment	10	0.3	*(7)*
			21	0.5	
		observed on tomatoes purchased a retail and stored for several days, with chlorine dip (210-280 ppm, 10s)	10	0.5	
			21	0.5	
yeasts/molds	cherry, skin	observed on tomatoes purchased a retail and stored for several days, no chlorine treatment	10	0.3	
			21	0.3	
		observed on tomatoes purchased a retail and stored for several days, with chlorine dip (210-280 ppm, 10s)	10	0.3	
			21	0.3	
Listeria monocytogenes Scott A	cherry, skin	dip inoculation, 1 min, storage over 8-20d	10	0	
			21	0.5	
	cherry, skin	chlorine dip (210-280 ppm, 10s) then dip inoculation, 1 min, and storage for 20d	10	0.1	
			21	0.5	
	chopped	chopped and mixed with inoculum, stored for 8-20d	10	-0.2	
			21	-0.9	
	chopped	chlorine dip (210-280 ppm, 10s) then chopped and mixed with inoculum, stored for 8-20d	10	-0.1	
			21	-0.9	

Table 5.4 Removal of *Salmonella* from tomatoes by chemical compounds (*n*)

Tomato Attributes	*Salmonella* serotype	Removal Compound	Conditions	Log reduction (load)	Ref
red, round	*Salmonella* Typhimurium LT2	40ppm peroxyacetic acid	2 min treatment in dumptank (11.3 kg in 130L)	2.5	*(27)*
		water		1.2	
		40ppm mixed peracid		2.5	
		40ppm chlorine		2.1	
		40ppm chlorine, acidified with citric acid		3.1	
		40ppm chlorine acidified with T-128		2.0	
		electrolyzed water with 40ppm chlorine		2.1	
mature, green	*Salmonella*	25ppm free NaOCl, acidified with HCl	8.5 log CFU/tomato; overhead spray with brush rollers, 5s, 15s, 30s, 60s	1.0, 2.0,2.5, 4.5	*(12)*
		50ppm free NaOCl, acidified with HCl		1.4, 2.8, 4.2, 5.0	
		100ppm free NaOCl, acidified with HCl		1.7, 4.0, 5.6, 5.5	
		water		1.4, 2.3, 2.5, 3.0	
		5ppm ClO_2		1.9, 3.5, 3.9, 4.9	
		80ppm peroxyacetic acid		2.8 4.7, 5.5, 5.5	
		100ppm free NaOCl, acidified with HCl	simulated flume treatment (10L), 5s, 15s, 30s, 60s	0.8, 1.3, 3.2, 3.3	
		water		0.5 1.0, 1.4, 1.3	
green, unwaxed	*Salmonella* (Angona, Gaminara, Michigan, Montevideo, Poona)	200ppm chlorine, pH 6.5	skin treatment (35C) 60s, 120s	3.6, 3.6	*(30)*
			stem scar treatment (35C) 60s, 120s	1.3, 1.5	
			puncture spot treatment (35C) 60s, 120s	1.2, 1.2	
		1200ppm acidified sodium chlorite (ASC), pH 2.5	skin treatment (35C) 60s, 120s	3.2, 3.2	
			stem scar treatment (35C) 60s, 120s	1.8, 2.0	
			puncture spot treatment (35C) 60s, 120s	1.2, 1.3	
		87ppm peroxyacetic acid	skin treatment (35C) 60s, 120s	3.7, 3.7	
			stem scar treatment (35C) 60s, 120s	1.4, 1.6	
			puncture spot treatment (35C) 60s, 120s	1.2, 1.2	
		Chlorine dioxide gas	skin treatment, 1h, 35C	2.2	
			stem scar treatment, 1h, 35C	3.8	
			puncture spot treatment, 1h, 35C	1.1	

Table 5.5 *Salmonella* contamination events associated with tomatoes (2004-2015)

Produce Item	Attributes	Microorganism	Contamination Event	No. Illnesses	Ref
Tomato	raw, large, red, round, whole and sliced	*Salmonella* Newport	consumed at restaurant; outbreak strain isolated from irrigation pond water near tomato fields in Virginia	72	*(10)*
Tomato	raw, Roma, chopped	*Salmonella* Braenderup	consumed at restaurants; diced and packaged in Kentucky; suspected source was animal feces and contaminated water in fields in Florida	82	
Tomato	raw	*Salmonella* Newport	consumed at restaurants; source not determined	115	
Tomato	raw, large, red, round	*Salmonella* Typhimurium	consumed at restaurant; single packhouse in Ohio supplied by 3 growers in 3 counties	190	
Tomato	red, round	*Salmonella* Javiana	consumed restaurant, home, daycare; source packinghouse in South Carolina	176	*(5)*
Tomato	red, round	*Salmonella* Montevideo	consumed at restaurant, home; source packinghouse in South Carolina	100	
Tomato	red, round	*Salmonella* Baildon	consumed at restaurant, nursing home; source not determined from farm in Florida	86	
Tomato	red, round	*Salmonella* Newport	consumed at restaurant, hospital, university, daycare; source on farm in Virginia	333	
Tomato	Roma	*Salmonella* Braenderup	consumed at restaurant; source not determined from farm in Florida	125	
Tomato	Roma	multiple *Salmonella* serotypes	consumed at restaurants; source not determined in any farm, packinghouse or fresh-cut facility in Florida, Georgia or South Carolina	429	
Jalapeño	whole	*Salmonella* Saintpaul	consumed at restaurants along with tomatoes; source from agricultural water on farm in Mexico but also found in distribution center in Texas and home in Colorado	1500	*(4)*

5.2.3 Working model

The working model of the PSCMT supply chain was optimized to obtain estimated values (log CFU) within one standard deviation of the observed aerobic plate count data from Chapter 2. The final parameters in Table 5.6 are a combination of literature values and those obtained from control tomatoes (log reduction) and statistical analysis (growth rate, Chapter 3). Distance to the distribution center and retail directly impact the microbial flow, so the shortest observed supply chain was chosen as the baseline. Introduction of contamination at subsequent postharvest handling steps was included and shown to partially explain the observed levels of aerobic plate counts (Chapter 3). The output of the baseline or working model is shown in Figure 5.2.

Table 5.6 Supply chain nodes, conditions and parameters for PSCMT simulation of observational study aerobic plate count data (Chapter 2)

Supply Chain Step	Node type	Conditions	Parameters	Reference
harvest	contamination	duration: 1 period	load: 100 (2 log)	*(34)*
holding	growth	dwell time: 8 per temp: 25°C	growth rate: 0.3	*(7, 34)*
spray wash	removal	dwell time: 1 per	log reduction: 0.5	*(7, 34)*
brush roller	transfer	segments: 2 clean: 1x/day	alpha: 0 beta: 0	*(28)*
dry roller	transfer	segments: 3 clean: 1x/day	alpha: 0.05 beta: 0.01	*(28)*
packing	growth	dwell time: 2 per temp: 25°C	growth rate: 0.3	*(7, 34)*
	contamination	duration: 4 per	load: 10	*(34)*
distribution	growth	dwell time: 144 per temp: 10-12°C	growth rate: 0.3	*(7, 34)*
retail	growth	dwell time: 96 per temp: 25°C	growth rate: 0.3	*(7, 34)*
	contamination	duration: 4 per	load: 100	*(34)*

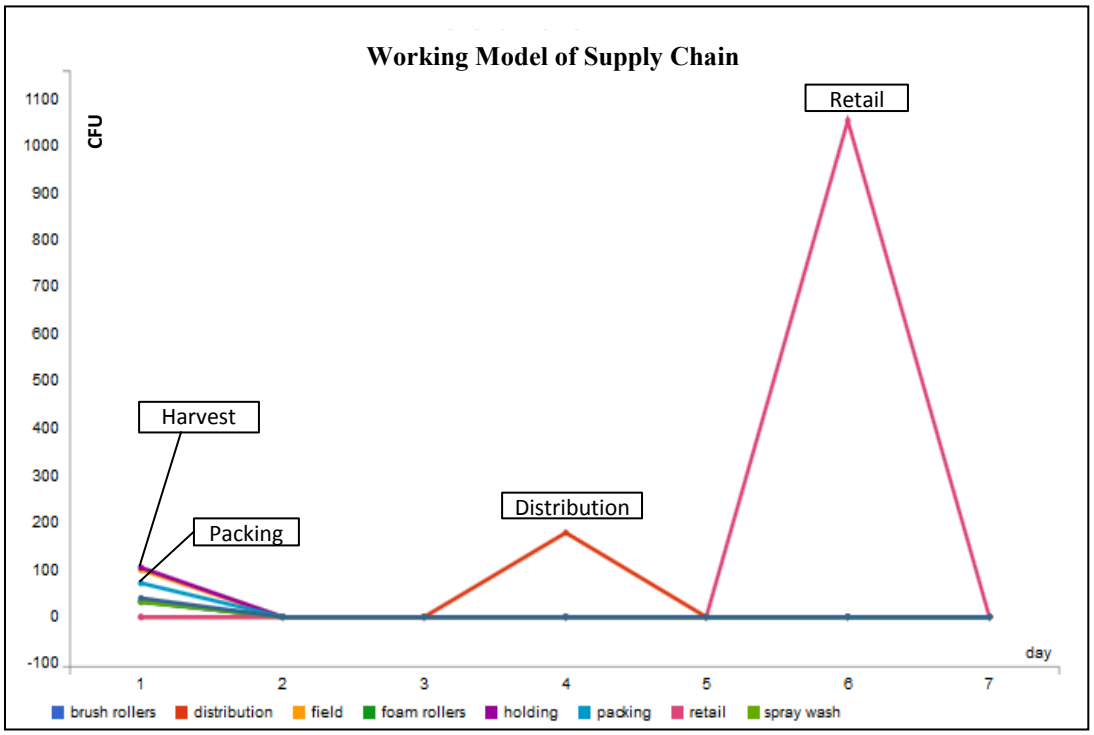

Figure 5.2 Output of the PSCMT representation of supply chain from Chapter 2 according to parameters in Table 5.6. The input value of 2.0 log CFU at harvest results in 1.9 log at packing, 2.3 log at distribution, and 3.0 log at retail compared to observed values of 1.9±1.1, 1.7±1.1, 2.3±1.1, and 3.5±1.4 log CFU, respectively.

With a representative model of the supply chain, further assessment of outbreak scenarios and interventions was possible, given the existing research on relevant parameters. Specifically of interest was testing of different contamination events, temperature abuse, sanitizer and packinghouse conditions, and the parameters relevant to *Salmonella* spp., among others which are reviewed below.

5.2.4 Effect of packing methods

It has been hypothesized that cross-contamination during washing and packing of fresh produce is an important source of risk for foodborne illnesses *(13)*. Different surface materials are used to transport, contain or handle products and the methods and frequency of cleaning these surfaces are related to the level of associated risk.

Furthermore, adequate levels of sanitizer in wash water serve to reduce viable microorganisms in the recirculated or reused water. For fresh tomatoes, there are three main packing methods to evaluate: packing the product directly after harvesting without washing or use of a packinghouse (field packing); washing and transporting the product with tanks and flumes of water (dump tank); and, washing and transporting the product with overhead spray and brush rollers, respectively (spray washing). In the model supply chain studied here, spray washing was used; however, it was of interest to study how different packing methods may alter the same microbial flow. Research on transfer parameters (alpha and beta) is given in Tables 5.1-5.2 and the parameters selected for simulation in the working supply chain are listed in Table 5.7. In summary, transfer for field packing takes place between tomatoes and cloth that is used to remove any soil during packing. Transfer for the dump tank method takes place in the water used. When it is adequately chlorinated, there is no transfer between water and tomatoes (β=0). Lastly, transfer during spray and brush washing may occur between tomatoes and the brush rollers, however a previous study has shown the transfer parameters for that system to be zero.

Table 5.7 Model parameters for effect of packing methods simulations

Location in Supply Chain	Field Packing	Dump Tank		Spray Wash	
		water	chlorinated	water (baseline)	chlorinated
Harvest	n = 100				
Holding	8 per, g=0.3				
Removal	n=0	n= 1.2	n= 3.1	n= 0.5	n= 2.8
Transfer	α= 0.02, β=0.03	a= 0.02, b=0.01	a= 0.02, b=0.0	a= 0.0, b=0.0	a= 0.0, b=0.0
Packing	--	2 per, g=0.3; contamination: 4 per, n=10			
Distribution	144 per, g=0.3				
Retail	96 per, g=0.3; contamination: 4 per, n=100				

5.2.5 Initial contamination events

The genetic makeup of *Salmonella* spp. is primarily encoded for pathogenesis and survival. Although its reservoir is the intestinal tracts of humans and animals, especially poultry, wild birds, reptiles, and amphibians, *Salmonella* has been demonstrated to persist in water, soil and the farm environment for several weeks to years, respectively, depending on the favorability of conditions *(6)*. One type of initial contamination event was modeled as a persistent source of contamination in a field. The other type of initial contamination event was a point-source of contamination. Point-source contamination could include an individual worker who was sick or not following proper hygiene practices on a given harvest day, a flood event, a wild animal running through a field. Point-source contamination was simulated for 1 period, while persistent of contamination in the production environment was simulated over 240 periods (or 5 days). Two log reduction values were tested for both contamination methods, 0.5 *(34)* and 2.8 *(12)*, to demonstrate the impact on removal operations. The reliability or resiliency of the supply chain was evaluated using several metrics: cumulative input from harvest; cumulative through the packinghouse and time; cumulative output at retail and time; and, maximum level at retail. Levels are given in $\log_{10}$CFU.

5.2.6 Evaluating growth rate on tomatoes and food contact surfaces

Growth is simply characterized by an exponential growth model according to daily growth rate (gamma) and dwell time. As either of those parameters increase, the impact of the growth node on the microbial flow increases. Conditions that alter the growth rate include wounding the tomato surface *(29)*, exposing product to higher

temperatures *(33)*, and chopping the tomatoes at retail *(2, 33)*, for example. Temperature fluctuations during transportation of fresh produce from field to distribution centers to retail have been measured and are significant *(31)*. These conditions and respective gammas are summarized in Table 5.3. In testing these effects, the baseline gamma of 0.3 at the location of growth was replaced with the appropriate gamma: wounded, 4.6; temperature abuse, 1.7; and, retail chopping, 4 periods, 4.0-9.4.

Salmonella has also been shown to survive, but decline, for up to 28 days on food contact surfaces used in packinghouses *(1)*. Both a gamma and a cleaning parameter were included in the transfer function to account for this behavior and the frequency with which surface contamination was removed. The effect of this gamma was studied on individual transfer nodes with 4 segments, with and without cleaning, for select materials listed in Table 5.8 with persistent and point-source contamination events. Materials with no estimates for alpha and beta were not used in simulations. The output measured was cumulative flow from the transfer node.

Table 5.8 Calculated gamma for *Salmonella* on packinghouse surfaces *(1)*

Material	30°C/80%RH	20°C/60%RH
Stainless steel	-1.0	-0.7
conveyor	-3.2	-0.9
polyvinyl chloride (PVC)	-1.2	-0.8
sponge rollers	-11.5	-1.6
unfinished oak wood	-1.2	-0.3

5.2.7 Effect of sanitizer concentration

Removal of contamination is defined by a log reduction and dwell time depending on the compound and method of application. Table 5.4 summarizes several

studies on sanitizer efficacy for removing *Salmonella* from tomatoes via different delivery systems. Particularly relevant to the supply chain modeled here, was the study utilizing the spray washing system *(12)* with different levels, application times and types of sanitizers. Although the baseline model used a 0.5 log reduction during the spray wash with chlorine (150 ppm total chlorine), Chang and Schneider *(12)* showed that both water and 25 ppm free chlorine delivered a 2.0 log reduction in 15 seconds of spray/brush washing. The effect of different sanitizers depends on concentration and application method. The reductions summarized here should be reviewed under the experimental conditions tested, and their use in a supply chain simulation may depend on the level of control required/desired.

5.2.8 Estimating supply chain length

Few studies report actual timing or logistics of packing, distribution and retail for fresh tomatoes or other produce commodities. The observational study in Chapter 2 provided examples of time-steps for a 10 day supply chain from Mexico to the USA. Zhou et al. *(32)* studied harvest and packing operations in Florida in detail and Le Strange et al. *(17)* has reviewed fresh-market tomato production in California. Harvesting usually occurs several times (but not daily) per week, followed by transportation to packing within 1-5 hours, but occasionally longer depending on the harvest. Tomatoes produced in Florida are commonly held for a number of days (3-9) in ripening rooms prior to packing, in which conditions are 20-21°C, 85-95% relative humidity and fruit are typically treated with gaseous ethylene. During 4-8 hours of packing, tomatoes are washed, sorted by size and maturity, and either bulk packed or packed in cardboard flats. Washing methods include either dump tank and flume

systems or spray and brush roller systems, both utilizing sanitizers in the wash water to prevent cross-contamination of pathogens while minimally impacting fruit quality. Depending on the capabilities of the packinghouse, product leaves on refrigerated trucks the same day it is packed to arrive at a distribution center or shipping point. From this point, the supply chain locations and distances become less clear as fresh-market tomatoes are sold via retail and food service marketing channels depending on their size, maturity and grade instead of traditional first-in-first-out procedures.

In this study, all simulations were run with 48 periods per day (30-minute time-steps). Harvest and packing occurred on the same day, however, were separated by a four hour holding time. For the baseline scenario, distribution and retail occurred over three and two days, respectively. It should be clear that the longer dwell times in growth nodes with positive growth rates (distribution and retail) will continue to increase the microbial flow. Similarly, addition of more nodes, other than removal, will spread the microbial flow over more days of the supply chain. Therefore, the effect of length of the supply chain was not specifically modeled but used in developing a relevant working model for other simulations.

5.3 RESULTS AND DISCUSSION

5.3.1 Effect of packing methods

The type of packing system utilized was modeled to compare recommended practices with the spray wash system used in the working supply chain. Also shown were the effects of removal and cross-contamination during packinghouse operations. When microbial flow was removed during packing, the overall output at retail was lower (Table 5.9). Specifically, in the field packing method, there was no removal

between harvest and packing and this grew to 3.7 log at the point of sale, the highest among all methods.

The dump tank and spray washing scenarios included removal and transfer operations, with and without chlorine. Mainly, chlorine increased log reduction and eliminated transfer in the dump tank. When chlorinated water was used *(12, 27)*, there was no difference in cumulative levels from the dump tank and spray wash packing methods (Table 5.9). As the transfer coefficients did not vary greatly from surface material to surface material, cross-contamination was not noticeable compared to direct contamination and removal events. In fact, high transfer coefficients from product to surface (alpha) and little or no transfer from surface to product (beta) suggested the transfer step was more similar to a removal process. Indeed the more probable effect of cross-contamination is on prevalence of products contaminated, instead of the level (Snary, 2016).

Table 5.9 Log microbial flow at locations along the supply chain simulated with different on-farm packing methods

Location in Supply Chain	**Field Packing**	**Dump Tank**		**Spray Wash**	
		water	chlorinated	water (baseline)	chlorinated
Harvest	3.0	3.0	3.0	3.0	3.0
Packing	3.0	2.0	1.6	2.6	1.6
Distribution	3.4	2.4	2.0	3.0	2.0
Retail	3.7	3.1	3.0	3.4	3.0

5.3.2 Effect of initial contamination event

Point-source contamination events showed more transfer between surfaces in the packinghouse, depending on the level introduced, and more cumulative flow due to the overloading of the removal function (Table 5.10). The persistent contamination

scenarios presented less contamination in each period, allowing for the removal step to be more efficient when adequately chlorinated. For example, comparing the baseline to the point-source for both removal values, microbial levels were present at packing for 22 and 52-53 periods following contamination, respectively. In other words, even after contaminated product had moved through the system, uncontaminated products became contaminated from residual levels in the packinghouse. Comparing the point-source to persisting contamination with the 0.5 log reduction showed the same number of periods for residual contamination in the packing house. However, when the log reduction was improved to 2.8 log, the cross-contamination from the persisting source was reduced to 16 periods of microbial flow from packing following the end of the contamination event. This does not necessarily alter the overall level, as seen in the cumulative log microbial flow through the packing operation, but would increase prevalence of contamination within a lot of produce.

The difference between the point-source and persisting contamination events in their spread of contamination over several days of the supply chain is illustrated in Figure 5.3. Additionally, using the cumulative output from retail and the maximum level at retail in a period indicate the differences in microbial flow between contamination events. For the point-source contamination, the cumulative and maximum levels at retail were the same, while for the persisting contamination the cumulative was greater than the maximum level at retail. All scenarios reached the maximum at retail around the same period, 256-260, due to the introduction of contamination at retail (Table 5.6).

Table 5.10 Results of initial contamination event simulations from working supply chain model

Contamination Metric	**Baseline** (Table 5.6 and Figure 5.2)	**Point Source** (1 per, 6 log, 0.5 log reduction)	**Point Source** (1 per, 6 log, 2.8 log reduction)	**Persisting Contamination** (240 per, 4 log, 0.5 log reduction)	**Persisting Contamination** (240 per, 4 log, 2.8 log reduction)
Cumulative In (Harvest)	2.0 log	6.0 log	6.0 log	6.4 log	6.4 log
Time clear from Packing	per 22	per 53	per 54	per 293	per 256
Cumulative Out (Packing)	1.9 log	5.5 log	3.2 log	5.9 log	3.6 log
Time clear from Retail	per 262	per 293	per 293	per 533	per 496
Cumulative Out (Retail)	3.0 log	6.2 log	3.9 log	6.5 log	4.3 log
Maximum at Retail	per 260, 2.4 log	per 256, 6.2 log	per 256, 3.9 log	per 260, 4.2 log	per 260, 2.5 log

Point-source and persisting contamination events may present different foodborne illness outbreak patterns. For example, recurrent outbreaks attributed to produce grown in the same region or even field, such as the *Salmonella* Newport outbreaks from tomatoes grown in the mid-Atlantic coast of the USA, suggested that the source in that production environment persisted over time *(15)*. On the other hand, norovirus is the most common cause of foodborne illness in the USA and is most often attributed to an instance of poor hygiene and handling practices at retail *(20)*, usually resulting in more isolated cases. While the simulations presented here involved point and persisting contamination from the field, the impact on the retail contamination level and the differences in dynamic behavior were illustrated.

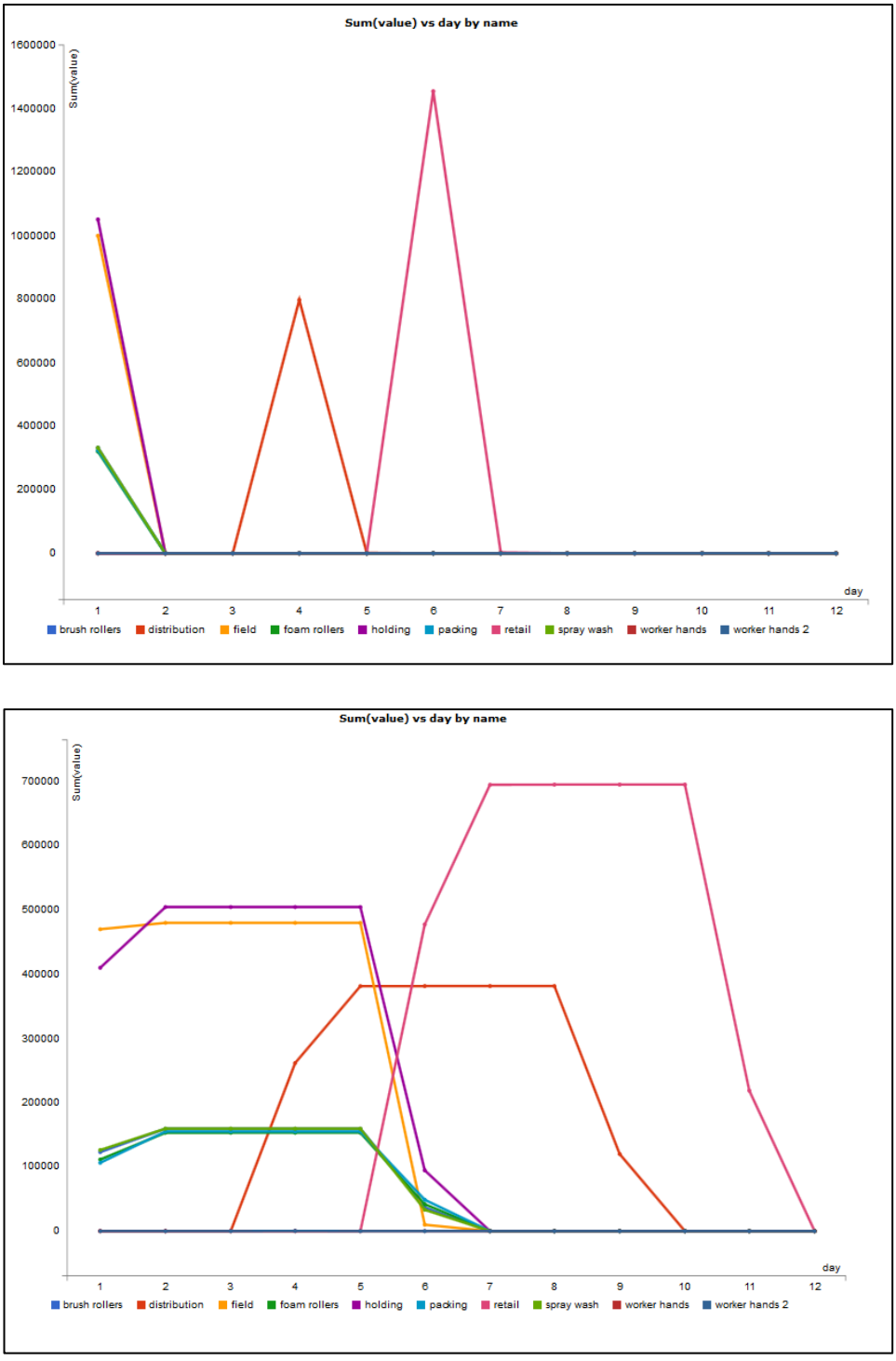

Figure 5.3 Output of PSCMT simulation of different initial contamination events. The top figure is the point-source contamination and the bottom is the persistent contamination, both with 0.5 log reduction.

5.3.3 Effect of growth rate on tomatoes

If product is wounded during harvest, the split tomatoes provide nutrients for growth of surface contamination. Furthermore, the holding step prior to packing allows time for this growth to occur and reach higher levels prior to washing, compared to unwounded produce. For example, with the same 2.0 log contamination event but growth for 8 periods due to wounding (γ=4.6), the level of microorganisms entering and leaving packing was 2.3 and 2.0 log, respectively (Table 5.11). When holding was extended for 16 periods, the 2.0 log from the field, grew to 2.7 log during holding and was reduced to 2.3 log during packing (data not shown).

When extreme temperature abuse (30°C) occurred during distribution, the same 2.0 log after harvest and 1.9 log from packing increase to 4.1 log and 4.4 log at distribution and retail, respectively, compared to 2.3 and 3.0 of the baseline (Table 5.11). This scenario showed the greatest increase due to the length of time the product was held in the distribution node. Temperature abuse at retail for 96 periods increased the level by 1.2 log compared to the baseline. Lastly, when the 3.0 log flow at retail was exposed to chopping and sale for 4 periods, the level increased by 0.2-0.4 log, depending on the reference used.

Table 5.11 Log microbial flow as a result of increased growth at different locations of the working supply chain model

Location in Supply chain	Baseline (Fig 5.2)	Wounded at Harvest	Temperature abuse at Distribution	Temperature abuse at Retail	Retail Chopping
Harvest	2.0	2.3	2.0	2.0	2.0
Packing	1.9	2.0	1.9	1.9	1.9
Distribution	2.3	2.4	4.1	2.3	2.3
Retail	3.0	3.1	4.4	4.2	3.2-3.4

5.3.4 Effect of surface materials and cleaning frequency

As transfer is very small, the output in Table 5.12 is given in cells instead of log values to demonstrate the subtle differences in the effect of using these materials. Alpha describes the fraction of microbial flow that is deposited on a surface material in each segment of the transfer function. In general, larger alpha values removed more cells from the flow and resulted in less cumulative output (i.e., sponge rollers). However, the sponge rollers also had the largest gamma value, indicating fewer microorganisms were surviving on the surface in each period. This faster die off compared to the other surfaces may have contributed to the lower transfer and lower microbial output across persistent and point source contamination events.

Beta describes transfer from the contaminated surface to the microbial flow (or product) and in the simulations essentially determined how long contamination remained in the transfer function and how much entered the flow. As research on beta was not available or consistent for all of these materials, two values were selected to test a range of transfer and the effect for each material. In general, the larger beta value, 0.07, resulted in fewer periods of contamination output and a higher cumulative flow out of the transfer, compared to β=0.01 across all materials, cleaning frequencies and contamination events. This was consistent with the exception of the conveyor with cleaning and point-source contamination, in which the periods of cross-contamination were slightly greater for beta of 0.07.

The same proportion of cumulative output to cumulative input in the microbial flow was the same for both the persistent and point-source events within a surface material, beta value and cleaning frequency. This was due to the nature of the transfer

function and the levels of contamination tested. The alpha parameter transfers a proportion of the 1000 cells introduced to the surface in a period and the beta parameter transfers a proportion of this surface level to the incoming cells in the next period. So, since the alphas and betas were the same, as well as the level of contamination in a period, the proportion of output to input was the same. Lastly, cleaning the surface once per day, shortened the periods of cross-contamination and cumulative output across beta values and contamination events, with the exception of the conveyor of beta 0.07 and point source contamination, in which cleaning made no difference.

Table 5.12 Cumulative output from packinghouse operations due to use of different surface materials and cleaning frequencies

Material	Transfer Parameters		Cleaning Frequency	Persistent (48 periods, 1000)	Point-source (1 period, 1000)
conveyor	α=0.02, γ= -0.9	β=0.01	no cleaning	per 254, 46139	per 120, 961
		β=0.07		per 144, 47390	per 51, 987
		β=0.01	once per day	per 96, 45609	per 49, 956
		β=0.07		per 90, 46860	per 51, 987
polyvinyl chloride (PVC)	α=0.03, γ= -0.8	β=0.01	no cleaning	per 273, 45340	per 215, 945
		β=0.07		per 129, 47168	per 74, 983
		β=0.01	once per day	per 96, 44461	per 49, 935
		β=0.07		per 93, 46341	per 45, 981
sponge rollers	α= 0.05, γ= -1.6	β=0.01	no cleaning	per 213, 42652	per 143, 889
		β=0.07		per 124, 45689	per 60, 952
		β=0.01	once per day	per 94, 42003	per 48, 884
		β=0.07		per 92, 44723	per 47, 951

5.4 CONCLUSION

Validation of the PSCMT model with observational study data provided a working model framework for testing components of a supply chain that contribute to level and duration of microbial flow in different locations. The efficacy of packing

methods showed that with distribution and retail fixed, the level of microorganisms leaving the packinghouse contributed to the levels at the end of the supply chain, as no other interventions are typically applied. Adequate chlorine levels provided similar control across packing methods. Further simulations focused on possible impacts along the supply chain: Initial contamination with a persisting field source increased spread over the length of the supply chain, while point-source contamination overloaded the removal operation and resulted in higher maximum values; Controlling product quality and the temperature of the distribution chain could prevent or slow growth of microorganisms by between 0.1-1.4 log; Surface materials used in packinghouse operations should be evaluated for alpha beta values and used along with regular cleaning and sanitation, but are consistent across contamination event types.

While this modeling tool provides data that may be incorporated in an exposure assessment, emphasis should be placed on the relative changes in contamination due to interventions. Insight into the microbial dynamics may provide produce growers, handlers and retailers some direction in focusing risk mitigation strategies. As data used for parameters in this model are taken from a wide range of studies, future work to incorporate uncertainty and variability will be critical in providing more confident microbial flow estimates. Additional supply chain studies of similar or different fresh produce commodities may be of interest (i.e. melon and *Listeria*, leafy greens and *E. coli*) for application of PSCMT modeling tool.

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CHAPTER 6

CONCLUSIONS AND PROSPECTUS

6.1 CONCLUSIONS

The risk of microbial contamination along the fresh produce supply chain threatens both public health and its reliability. For example, health-related costs from the 22 million people affected by foodborne illness from fresh produce each year in the USA have been previously estimated at $38 billion *(3, 12)* and the impact of outbreaks in fresh produce on consumer confidence and purchasing habits has been shown *(13)*. While the supply chain is specifically designed to both preserve and monitor attributes of the product and its production environment, fresh produce is grown, harvested, handled and sold in highly variable environments.

There is a body of research on prevalence of pathogens and contamination risks during production and harvest *(2)*, cross-contamination in cutting and packing *(1, 5, 6, 9-11)* (and Tables 5.1-5.2), along with temperature fluctuations during storage and distribution *(14)*, but the rare occurrence of pathogens and outbreaks makes these events difficult to study and prevent. Understanding microbial dynamics in supply chains may facilitate continued research and prevention strategies. Risk assessment models and tools allow for simulation of potential microbial contamination behavior and have been developed for a variety of food commodities, reviewed in Table 1.2. There were several limitations when applying these tools to the fresh produce supply chain, prompting further research in these areas: data collection in all locations of the supply chain; the effect of minimal handling events postharvest on microbial populations; spread of contamination through a non-linear network of nodes.

The observational study provided proof-of-concept that microbial dynamics existed on product moving through a supply chain, compared to products held in controlled conditions. Aerobic plate count, total coliforms, generic *E. coli*, and yeasts and molds were selected as indicator organisms to represent microbial populations associated with safety and quality. Specifically, increasing levels and variation at handling points may have indicated more variable microbial conditions and the opportunity for improved practices. Estimation of the means and standard deviations of these indicator microorganism populations on tomato surfaces allowed for further statistical analyses on characterizing these population changes in terms of prevalence and concentration. Traceability of products from the field to retail provided qualitative data for design of a postharvest risk model.

Logistic and linear regression models were used on the microbial count data to study supply chain effects on prevalence and concentration, respectively. Location practices were found to influence the cross-contamination and spread (prevalence) of microbial populations from products to surfaces, and vice versa, while time in the supply chain impacted the concentration of a microbial population on the product. More specifically, locations with increased prevalence of microbial indicators of food safety importance were the packinghouse and retail market and the difference in concentration between a six-day supply chain and a ten-day supply chain was often 2 log CFU/g. These findings suggested that practices in the packinghouse and retail environment should focus on limited handling and frequent cleaning and sanitation of food-contact surfaces, and that the design of shorter and more efficient supply chains may limit microbial growth on products.

A user-oriented tool, Produce Supply Chain with Microbial Travelers (PSCMT), employed a dynamic simulation program based on detailed deterministic equations that describe microbial behavior to estimate how the concentration parameter changes due to postharvest handling and operations. PSCMT's flexibility allowed for simulation of microbial behavior in a network of supply chains and its transparency provided the opportunity to understand how the parameters chosen affect overall population behavior, including contamination type, packing method and materials used. The output given as either number of microorganisms, log microorganisms or concentration in the Pivot Chart can further help in visualizing optimal intervention strategies along the supply chain. Construction of this mechanistic model provided a novel method for conceptual understanding of such transmission dynamics as a result of a given fresh produce postharvest system design.

6.2 PROSPECTUS

The author collected data and estimated parameter values for indicator microorganisms on fresh tomatoes moving through a supply chain from Mexico to the USA, which was necessary for development of a novel modeling framework for risk assessment in the postharvest supply chain of fresh produce. This provided a proof-of-concept for further observational studies and improvements to the PSCMT modeling tool. The following are suggested for future work:

Quantification of microbial indicators on produce in the supply chain. The observational study conducted in Chapter 2 involved one supply chain of one fresh produce commodity during one harvest season utilizing protected agricultural systems. As there are few studies reporting quantified microbial data (indicators and pathogens)

at different locations of the supply chain, external validity of these results is needed. Sampling will have to be repeated in another harvest year, and other growers, distributors and retailers should be recruited. It may be interesting to sample farms utilizing other production practices, such as open fields, to compare microbial levels at harvest or to compare a supply chain with less control. Larger sample sizes taken from lots and more consistent sampling methods at retail will provide more confidence in prevalence estimates. It is not recommended to analyze produce samples for microbial pathogens or to relate indicator data to the prevalence of pathogens in the environment.

Design of a stochastic model with prevalence. In its current state, several aspects of quantitative microbial risk assessment (QMRA) and similar models remain out of the scope of the PSCMT modeling tool. For example, the model is purely deterministic, so there is no stochasticity for evaluating variation or uncertainty of model parameters. As parameter estimates are taken from numerous studies, most of which do not provide similar values, incorporating probability distributions for parameters will allow for true simulations and confidence intervals around microbial flow levels along the supply chain. Similarly, PSCMT is not alone a complete QMRA, but merely an exposure assessment as a preliminary step in the QMRA process. The output of the model could be an input into existing dose-response modeling tools, if the risk of illness is desired. Lastly, as the model simulation of concentration is already complex and does not incorporate stochasticity, prevalence is not an included attribute of the product flow. While concentration is a good starting point for risk assessment, as prevalence may be estimated from concentration *(4)*, prevalence estimates will

complement the assessment of spread of contamination through a supply chain, especially at points of fractionation and joining *(7)*. Prevalence has been included in stochastic, compartmental models for processed food and cut produce *(1, 11)*, but not yet fresh produce supply chains.

Assess model validation and adequacy. The PSCMT model structure and parameters were verified by frequent debugging and use of the vast literature summarized in Chapter 5. Validation of the model was also conducted in Chapter 5 on the observed aerobic plate count data in Chapter 2. Additional assessment of the model validity and adequacy for fresh produce supply chains is needed. Data from existing or future supply chain sampling studies will be needed to calibrate model parameters for the different nodes. Additionally, assessment of the adequacy of the model for representing fresh produce supply chains and associated risks should be conducted by soliciting expert opinions or by hosting the tool on a server receiving feedback.

Alternative applications of PSCMT. The PSCMT model attributes were demonstrated in Chapter 5 on the postharvest supply chain of fresh tomatoes and the impact of applying control in different areas. If prevalence is implemented in a future version of the model, use of the tool for sampling and detection calculations would be beneficial to scientists, regulators and industry, in design of experiments, focusing resources, and applying controls, respectively. Alternative applications of the tool exist as a complement to future risk assessments for other risky produce commodities and their unique handling operations, such as melons, leafy greens, sprouts or herbs *(8)*. In production fields, it is recommended by the FDA to allow for a 0.5 log/day

die-off in soils that have been irrigated with contaminated water. This could present an interesting management decision to model its efficacy of control for different contamination events. Focusing in packinghouse or food production facilities, the modeling tool could be used to better understand or conceptualize the movement of contamination from niches in “zone 4” (non-food-contact) to product in “zone 1” (food-contact surfaces).

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